

# **RIFT VALLEY FEVER VIRUS WORKING GROUP**

**24-26 August 2004**

## **Summary Report and Recommendations**

**ANSER**

**Arlington, VA**

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## Executive Summary

This report supports the United States Department of Agriculture Animal and Plant Health Inspection Service (APHIS) Project “Vulnerability and Response to Agroterrorism: Protecting America’s Agricultural Infrastructure and Economy From Emerging Threats” and is the result of a combined effort to produce scientific and technical reports on subjects related to bioterrorism and other biological threats to farm animals.

In May 2004, a Farm Animal Infectious Disease Research Core Group met to identify and prioritize leading biological threat agents and select one for investigation by a working group (WG) of renowned U.S. and international subject matter experts. For the leading biological threat agents, the Core Group weighed the economic and public health implications of an introduction of specific agents; the availability of diagnostic, preventive, and control options for the diseases caused by these agents; and the current state of agent- and disease-related research. The WG’s prioritization of the principal biological threat agents was also guided by recent studies by other expert panels assessing bioterrorism threats to U.S. food animal populations.<sup>1</sup> After carefully considering all of the above criteria, the Core Group selected Rift Valley fever virus (RVFV) as the initial agent to be evaluated and identified three key researchable areas for consideration by the WG: (1) detection, identification, and diagnostics; (2) vaccines; and (3) epidemiology, control, and impact.

The Core Group found it valuable to compare RVFV to West Nile virus (WNV), another mosquito-borne, zoonotic virus that is now endemic to the United States ([Table 1](#)). Rift Valley fever (RVF) likely presents a much greater public health and economic threat to the United States.

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<sup>1</sup> “Strategic Research Targets to Protect American Livestock and Poultry From Biological Threat Agents,” report from the WMD Counter Measures Working Group, Animal Pathogen Research and Development Subgroup, 31 October 2003; NIAID Biodefense Research Agenda for CDC Category A Agents, February 2002; HHS/USDA Overlap Select Agents, 2002; “HFV as Biological Weapons,” Working Group on Civilian Biodefense Consensus Statement on Biological Weapons, *Journal of the American Medical Association*, 2002, 287:2391-2405.

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|  | <b>West Nile Virus</b>  | <b>Rift Valley Fever Virus</b>  |
|--|---|---|
| Disease type   | Flavivirus (zoonotic arbovirus)                                       | Bunyavirus (zoonotic arbovirus)   |
| Priority biological terrorism threat agent   | No  | Yes <sup>1</sup>  |
| Considered for past biological warfare use   | No  | Yes (U.S. <sup>2</sup> , USSR <sup>3</sup> )  |
| HHS/USDA overlap agent   | No  | Yes   |
| OIE trade restrictions apply   | No  | Yes   |
| Endemic to U.S.  | Yes   | No  |
| Vectors endemic to U.S.  | Many mosquito species, including genera <i>Culex</i> and <i>Aedes</i> | Many mosquito species of <i>Culex</i> and <i>Aedes</i>                                  |
| Demonstrated transmission  | Infected mosquitoes   | Infected mosquitoes; direct contact with infected blood/tissue; aerosols                |
| Animal species infected  | Birds, horses and humans  | Ruminants, rodents and humans   |
| Reported mortality rate  | 0.6% (human); ~40% (horses)   | Up to 13.9% (human) <sup>4</sup> ; >70% young ruminants; (abortions in ruminants ~100%) |
| Licensed U.S. animal vaccine <sup>4</sup>  | Yes   | No  |
| Licensed U.S. human vaccine <sup>4</sup>   | No  | No  |
| Sentinel surveillance in U.S.  | Yes   | No  |
| Rapid detection capability   | Yes   | No  |
| Diagnostic testing protocol  | Yes   | Yes   |
| U.S. live virus laboratory testing capability  | Commercial laboratories   | Biosafety Level-3 (Plum Island Animal Disease Center & USAMRIID)                        |
| Rate of spread in U.S.   | 48 contiguous states/48 mos.  | Unknown   |
| <sup>1</sup> "Strategic Research Targets to Protect American Livestock and Poultry From Biological Threat Agents," Office of Science and Technology Policy Blue Ribbon Panel on the Threat of Biological Terrorism Directed Against Livestock, Terrence K. Kelly, et al., CF-193-OSTP, April 2004, Prepared for the Office of Science and Technology Policy, p. 20.  |   |   |
| <sup>2</sup> The U.S. biological program studied RVF. Source: Stockholm International Peace Research Institute, <i>The Problem of Chemical and Biological Warfare</i> , Volume I: <i>The Rise of CB Weapons</i> , pp. 122-123.   |   |   |
| <sup>3</sup> U.S. Department of Justice, Office of Justice Programs, National Institute of Justice, <i>An Introduction to Biological Agent Detection Equipment for Emergency First Responders</i> , NIJ Guide 101-00, December 2001, p. 9.   |   |   |
| <sup>4</sup> Although lower mortality rates have been the norm in reporting, this value was obtained from the August–October 2000 <i>Morbidity and Mortality Weekly Report</i> , 13 October 2000 / 49(40); 905, "Outbreak of RVF—Saudi Arabia." While the reported values are likely a manifestation of the health and severity of disease within the population examined and the genotype of the virus, additional data, studies, and analysis are needed to predict possible mortality rates for a generally healthy but RVFV immunologically naïve population such as that which exists in the United States. |   |   |
| <sup>5</sup> Meets U.S. standards for efficacy and safety.   |   |   |

**Table 1—Comparison of the West Nile Virus and the Rift Valley Fever Virus**

The WG met at the Analytic Services Inc. (ANSER) facilities in Arlington, Virginia. This report captures its deliberations on important, relevant characteristics of RVF/RVFFV and highlights the findings of the WG along with its key concerns and recommendations.

There are several reasons why the virus and disease merit immediate attention:

- Introduction of RVFV to the United States would severely disrupt the beef cattle and dairy industry and cause more significant human illness and likely greater mortality than WNV.
- There exist critical gaps in our capability to detect, respond to, contain, and eradicate RVFV and RVF.
- RVFV is recognized as a potential biowarfare/bioterrorism agent.
- The range of natural transmission of RVFV has been steadily increasing. The first cases of RVF outside of Africa took place in 2000.
- Regardless of how RVF may be introduced, the nature of its vector-borne epidemiology means that if it is not rapidly contained it would spread as rapidly as WNV, but with far more serious consequences.
- Decision makers are generally unaware of the significant threat the virus and disease pose to the United States.

## Key Issues

- Generally, RVF detection in animal and human populations is problematical, as veterinarians and physicians outside its endemic area are not predisposed to search for the virus as an etiological agent of the disease. An investigation of RVF outside of Africa, Saudi Arabia, or Yemen would not likely occur until the presence of acute symptoms in a significant number of animals or humans was observed.
- The most likely tip-off event for an RVFV infection would be an “animal abortion storm” in a region with favorable climatology for the principal vector.
- Only limited testing and identification for the virus and disease are currently available.
- There is no one single method for detecting and identifying RVFV. A supporting laboratory program must have the ability to use classical methodologies, such as viral growth and serology, pathological procedures (for example, immunohistochemistry), and modern molecular diagnostic techniques, including reverse-transcriptase polymer chain reaction.
- Other strategies for detecting RVFV, other than direct laboratory testing of infected blood or tissue, include establishing electronic reporting networks for animal abortions, the use of disease and environmental modeling, and expanding the existing multiplex vector-sampling programs to include RVFV.
- Vector control will play a major part in RVF epidemic suppression. However, most cattle production states have no local mosquito control organizations.
- No studies have been done on the susceptibility of various species of North American wildlife to RVFV or how best to control or treat an event within the wildlife population.
- There is no fully established and comprehensive nationwide veterinary and public health awareness and training program associated with RVF that is readily accessible to all levels of health practitioners.
- There is no licensed commercial vaccine (that meets U.S. standards of safety and efficacy) currently available for RVF.

- The development of suitable candidate RVF vaccines within the U.S. will require significant investment from the Federal Government and the private sector, and in some cases the effective use of cutting-edge technologies will be required to support these efforts.
- Modeling and remote sensing can be used to design plans for the most effective vaccine and mosquito control response.

## Recommendations

1. A joint APHIS/CDC RVF cooperative response strategy and associated contingency plan need to be developed.
2. A full set of requirements for an emergency RVF vaccination program for the United States needs to be established. This effort should include a partnership with one or more international vaccine manufacturers.
3. Appropriate veterinary and public health training and awareness for RVF should be developed through USDA/CDC interagency cooperative arrangements.
4. The reporting and investigation of animal abortions, possibly utilizing already implemented human syndromic surveillance systems, must be improved.
5. Laboratory diagnostic tools and testing protocols for the virus and disease must be validated. Policies and procedures for their use must be established. Following the development of these policies and procedures, the systems should be made fully operational in a variety of venues.
6. Obstacles to full development of promising human and animal candidate vaccines must be overcome. A primary safe and efficacious RVF animal and human vaccine should be selected. To do so, further research, development, and evaluation of the human and animal MP-12 RVF vaccine, as well as promising alternative candidate vaccines, should be fully supported.
7. Once the best candidate RVF vaccine has been selected for full development, production and stockpiling should proceed rapidly. Concomitantly, plans for the effective distribution and policies for use of this vaccine must be firmly established.
8. A wide range of epidemiological disease models for the virus and disease needs to be developed, tested, and validated.
9. RVF control strategies should be thoroughly tested and evaluated.

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**Enclosure 1 (Compact Disk with four PowerPoint Presentations).** Topic 1 presentation: Dr. T. G. Ksiazek, “Rift Valley Fever Dx”; topic 2 presentation: Dr. C. J. Peters, “Rift Valley Fever”; topic 3 presentation: Dr. K. J. Linthicum, “Use of Remote Sensing in Formulating a Predictive Model for RVF in East Africa”; topic 4 presentation: Dr. J. M. Wilson, “Rift Valley Fever: Non-traditional Surveillance and Threat Assessment.”

## A. Introduction

### General Background

This report supports the U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS) Project “Vulnerability and Response to Agroterrorism: Protecting America’s Agricultural Infrastructure and Economy From Emerging Threats.” It is the result of a combined effort between ANSER and its partners at the Kansas State University National Agricultural Biosecurity Center (KSU-NABC) and BJE Associates, Inc., to produce scientific and technical reports on areas or subjects related to bioterrorism and other deliberate and inadvertent biological threats to farm animals.

In May 2004, a Farm Animal Infectious Disease Research Core Group, composed of representatives from the Agricultural Research Service (ARS) and APHIS, met to identify and prioritize the leading biological agents that posed the greatest threat to livestock agriculture and, as applicable, to humans. In this determination, the Core Group weighed the economic and public health implications of an introduction; the availability of diagnostic, preventive, and control options; and the current state of disease-related research. It was also guided by recent studies by other expert panels assessing bioterrorism threats to U.S. food animal populations and addressing the vaccine requirements needed to protect agricultural livestock against attacks by terrorists or others using biological agents.<sup>2</sup>

During its deliberations, the Core Group noted that the Rift Valley Fever Virus (RVFV) has a number of similarities to another zoonotic arbovirus of key interest and concern, the West Nile Virus (WNV). It concluded that the associated disease, Rift Valley Fever (RVF), presents significantly higher agricultural hazards, as well as troubling scientific challenges relating to disease diagnosis and protection. Subsequently, the Core Group selected RVF and its causative agent, RVFV, as initial subjects to be evaluated by a working group (WG) that was hand selected from a comprehensive list of renowned U.S. and international subject matter experts.

The Core Group identified three key researchable areas for consideration by the WG: RVF/RVFV (1) detection, identification, and diagnostics; (2) vaccines; and (3) epidemiology, control, and impact.

On 24, 25, and 26 August 2004, the WG met at the ANSER conference facilities in Arlington, Virginia, to thoroughly evaluate the three areas listed above. Dr. Barry Erlick served as the meeting facilitator. The attendees are listed in [Appendix 1](#).

The WG served as a forum for the subject matter experts to determine the completeness of scientific knowledge regarding the key characteristics of RVF/RVFV and to provide recommendations, specific actions, and related research efforts to address identified areas of concern. This report captures the key ingredients of deliberations, provides general background material on numerous aspects of RVF/RVFV, and highlights the findings of the WG along with key concerns and recommendations.

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<sup>2</sup> “Strategic Research Targets to Protect American Livestock and Poultry From Biological Threat Agents,” report from the WMD Counter Measures Working Group, Animal Pathogen Research and Development Subgroup, 31 October 2003; NIAID Biodefense Research Agenda for CDC Category A Agents, February 2002; HHS/USDA Overlap Select Agents, 2002; “HFV as Biological Weapons,” Working Group on Civilian Biodefense Consensus Statement on Biological Weapons, *Journal of the American Medical Association*, 2002, 287:2391-2405.

## Key Facts: Rift Valley Fever<sup>3</sup>

RVF is an acute, fever-causing, vector-borne, zoonotic disease. Like WNV, RVFV is a zoonotic arbovirus, spread through a mosquito vector. Cattle, sheep, and goats serve as the primary amplifier of the virus. Upon developing adequate viremia, humans may also serve as a virus reservoir for mosquitoes.

Abortion is often the only sign of RVF seen in pregnant adult animals. Although non-pregnant adults are susceptible to infection, they are more resistant to the clinical disease. The acute form is most common in young animals, where it causes high mortality. There is considerable variation in the susceptibility of animals to different RVFV genotypes. Animal breeds exotic to Africa, or from areas where RVF is not endemic, tend to be more susceptible.

RVFV was first isolated in 1930 in the Rift Valley of Kenya. The disease has since reached epidemic proportions, emerging irregularly in Kenya every 3 to 10 years. Extensive human disease events were not reported until 1951, when an estimated 20,000 persons were infected during an epizootic of cattle and sheep in South Africa. Reported RVF events came exclusively from sub-Saharan Africa until 1977-78, when approximately 18,000 persons were infected and 598 deaths were reported in Egypt. Ten years later, in 1997-98, there was a severe epizootic in East Africa that resulted in huge losses among the domestic animal population and significant human disease. This RVF event infected millions of cattle, sheep, and goats, as well as 89,000 humans. Over 500 human deaths were reported. In September 2000, RVF events were reported in Saudi Arabia and Yemen. These represented the first RVF cases identified outside Africa, demonstrating a significant potential for further spread. During the September 2000 event, Saudi Arabia experienced a reported 13.9% human mortality rate among the sick that were hospitalized.

RVF epizootics tend to occur periodically following heavy rains that flood natural depressions, creating reservoirs and allowing the primary vector (*Aedes* mosquitoes) to hatch. *Aedes* mosquitoes acquire the virus from feeding on infected animals and are capable of transovarial transmission (transmission of the virus from infected female mosquitoes to offspring via eggs) so that new generations of infected mosquitoes may hatch from their eggs. This provides a durable mechanism for maintaining the virus in nature since the eggs of these mosquitoes may survive for periods of several years in dry conditions.

A usual outcome of an RVFV infection in animals is a significant viremia that leads to virus amplification in the primary host, infection of secondary arthropod vector species and collateral transmission to humans. Transmission of RVFV to humans is also known to occur by aerosols of blood or amniotic fluid or by other direct contact with infected blood or tissue. Laboratory workers have displayed symptoms associated with the disease after working with infected blood and tissue samples. Human infection by RVFV vectors is a prominent feature in countries with a relatively small population of animal hosts. In these circumstances an RVF event may be recognized first in humans. Human infection manifests a broad spectrum of disease symptoms ranging from asymptomatic infection to a benign febrile illness to a severe illness that can include retinitis, encephalitis, and hemorrhagic fever. As noted in Saudi Arabia during the 1997-98 RVF event, the human mortality rate of patients presented may exceed 10% for a non-endemic population.

*Notes:* [Appendix 2](#) presents additional background information on general characteristics of RVF and zoonotic diseases, including pathogen threats to animal health, the potential impact of biological threat

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<sup>3</sup> Ibid., p. 16.

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agents, vaccine profiles and manufacturing, clinical features of RVF, differential and laboratory diagnosis, and the use of hemorrhagic fever viruses as biological weapons. An illustrative scenario outlining a potential RVF event in the United States and its impact is provided in [Appendix 3](#).

## B. Summary of RVF Working Group Discussion

### 1. Introduction

#### *Why Consider the Rift Valley Fever Virus?*

Many disease-causing agents such as *Bacillus anthracis* (anthrax bacterium), variola major (human smallpox virus), and WNV are well known to policy makers; however, RVFV is not. Decision makers are generally unaware of the potential impacts of an RVF event and the gaps in our capability to detect, respond to, contain, and eradicate the disease. Compared to the WNV, the introduction of RVF to the United States would have a significantly greater impact on public health, agricultural livestock, and the overall economy of the Nation and its bordering countries—a frightening possibility. The purpose of this WG was to determine whether the United States could effectively react to an intentional or natural introduction of RVF. To determine this, the WG identified what tools would be needed to identify, contain, and eliminate RVF from the United States.

#### *RVFV Has Been Known to Produce Hemorrhagic Fever*

Like WNV, RVFV is spread by mosquitoes. Unlike WNV, it is one of the 15 pathogens on the Office International des Epizooties (OIE) “List A”: diseases with a high potential for rapid spread, serious economic or public health consequences, and significant impact on the international trade of animals and animal products. The seriousness of this disease was also highlighted in the document “Strategic Research Targets to Protect American Livestock and Poultry From Biological Threat Agents,” which reported the results of an interagency WG investigating gaps in the United States’ capability to respond to various biological threat agents. In this document, RVFV was identified as a serious biological threat agent requiring scientific research. As a threat agent, RVFV has the potential for use as a biological warfare agent. Because this virus is non-endemic to the United States, this nation would be especially vulnerable. Coupled with a probable delay in detecting and identifying the disease, a naïve U.S. population could experience an explosive rate of infection in both animals and humans. Experts anticipate that with a clear understanding of the disease and proper scientific research, the economic and public health impact of an intentional or natural introduction of RVF into the United States can be controlled with minimal losses. Ideally, to protect the United States, it would be best to work with RVFV in locations outside the United States where the disease is endemic.

The WG was asked, “Do we have the capability to deal effectively with RVF, if and when it is introduced to the U.S.?” The answer was “No, we need to identify the tools and solutions that will rapidly give us the future capability to effectively deal with a RVF event.”

### 2. Topic 1 Discussion Notes: Detection and Identification

**Overview:** Presented by Dr. T. G. Ksiazek (see [Appendix 9](#) for an outline).

### ***History of Diagnosis***

- The virus was first identified in the 1930s
- Sheep were initially used for diagnosis. Mice and hamsters are now also used
- A wide variety of cell cultures is used to grow RVFV—for example, the CDC uses Vero-E6
- Mice were first used for virus neutralization assays; now cell culture is used

### ***Patterns of RVF Infection***

- The incubation period for RVF is relatively short (3-5 days in adult humans and animals and 12 hours in young animals)
- The fever coincides with a short-duration viremic phase
- The viremic phase is higher in humans than other affected species
  - This is 3-10 days compared to 2-5 days in cattle
  - The amplitude of the viremia is quite high
- This contrasts to other viral agents exhibiting a long-duration viremia
  - Lassa fever virus (delayed antibody response)
  - Hantaviruses (no viremia with the antibody already present)
- A long-lasting immune response
- A lifelong immunoglobulin G (IgG) and neutralization antibody response in humans

### ***Environmental Sensitivity of RVFV***

- It is very acid sensitive
  - For example, shortly after slaughter, the acidity of meat changes and is sufficient to kill the virus
  - Solutions having a pH of 6.2 or lower, including household disinfectants, are effective disinfectants
- Pasteurization of milk kills the virus

### ***Current Diagnostic Techniques and Test Selection***

- For diagnosis in the acute phase, use ELISA (enzyme-linked immunosorbent assay), immunohistochemistry, or reverse-transcriptase polymerase chain reaction (RT-PCR)
- For surveillance, use ELISA to detect immunoglobulin (IgM)/IgG.
  - The appropriate test depends on the phase of infection.
    - Identification of antigen is used during the viremic phase. This is a very sensitive method.
    - As viremia passes and antibodies increase, IgM detection is used

- Identification of IgG is used in an area where the virus is endemic to show that individuals who are very sick have been previously infected
  - Issues
    - Some acutely ill individuals have tested negative for IgG but were later demonstrated to be positive for antigen
    - In some cases, individuals tested negative for IgG initially but tested positive later in, for example, a third test

### ***Examples of Other Diagnostic Technologies***

- Molecular diagnosis
- Non-nested PCR
  - This works well because of the amplitude of viremia
- Nested amplifiers
  - This yields sequence data that can be used for phylogenetic analyses

### ***Current Diagnostic Capabilities and Limitations***

The U.S. experience with anthrax highlighted the vulnerability of having a single test center as the sole source of diagnosis. A high volume of samples can rapidly overwhelm a single laboratory. Plum Island Foreign Animal Disease Diagnostic Laboratory is the only agricultural BSL-3 laboratory in the United States with RVFV diagnostic capability. Additionally, for the initial diagnosis of a non-endemic exotic animal disease such as RVF, new virus must be isolated from laboratory-grown tissue culture cells inoculated with a sample of blood or tissue from a putatively infected animal and then characterized. Once a sample is submitted, it may very well take a week to isolate and identify the etiological viral agent.

It should be noted that following the initial diagnosis at Plum Island Animal Disease Center the required technology needed to make a rapid identification of the viral nucleic acid (RT-PCR), and thus the likely presence of RVFV, is generally available at the regional laboratory level. For this method of testing, however, RVFV nucleic acid primers and all extraction methods used must first be fully validated by APHIS. It is important to note that in the case of foot-and-mouth disease (FMD), the time from the decision to validate the RT-PCR testing procedures to an operational capability was two years. Therefore, now is the time to invest in the development cycle leading to a validated operational RVFV RT-PCR test.

### ***Detection of RVF***

The most difficult issue: “How will we detect RVF?”

Kenyan veterinarians regularly monitor designated sentinel farms for the presence of RVFV.

There was a wide-ranging discussion about the possibility of “piggybacking” on surveillance testing being conducted in the United States to detect the presence of WNV. Although there is no central U.S. surveillance network, many individual testing programs are in place. For example, California has a program to collect and test mosquitoes for the presence of a variety of viruses, and many states have programs that use crows as sentinel animals for WNV.

We should be able to add RVFV to current vector surveillance systems. While there are costs associated with the additional test reagents (such as those needed for ELISA testing), this approach will be less expensive than starting a new program from the ground up. Such an approach will save developmental time and will provide a surveillance capability where there is currently none. Additional cost-saving measures could include periodic testing (versus continuous) and geographically targeted testing.

The most likely tip-off event for an RVFV infection would be high abortion rates among livestock (an abortion storm) in a region where there is favorable climatology for the principal vector, mosquitoes. There would be clinical indications of fetal liver damage in the aborted fetuses and perhaps associated human illness.

An abortion-testing scheme would provide a chance to detect early signs of RVF (potentially the first case). More in-depth testing would be needed after confirmation of the disease.

Currently, over 50% of livestock abortions are never definitively diagnosed. With abortion being a key indicator of RVFV infection, it is critical that we lower this statistic and achieve a definite diagnosis of abortions. Veterinarians in the United States are not predisposed to look for RVF. To correct this, training to heighten awareness of RVF and an abortion-testing protocol to detect RVF must be provided.

The issue of detection in the human population presents the same problem as in animals. Physicians are also not inclined to look for RVFV. In identifying RVF, acute symptoms would be the likely cause for association with RVF, and the travel history of infected humans would be of key interest. Conversely, passengers traveling from regions where RVF is endemic may merit special screening. CDC currently performs only 20 tests for RVF per year, and this is usually on recent travelers from areas where the virus is endemic.

### ***An Approach to RVFV Testing in Animals***

- Develop an awareness and training program for breeders, veterinarians, and foreign animal disease diagnosticians.
- Establish parameters that would trigger a test for RVF when investigating animal abortions (for example, a fetus presents with necrotic liver).
- Integrate an RVFV testing protocol into the existing battery of tests used in regional labs and distribute testing supplies and reagents to regional labs.
- Possible technologies include RT-PCR and ELISA with IgM.
- RT-PCR is being pushed hard by the commercial entities that produce the technology. Additionally, regional labs are developing an RT-PCR testing capability.
- ELISA with IgM is already widely in use and less expensive than RT-PCR (the issue surrounding this procedure is which antigen to use).
- IgM testing would provide the broadest coverage in terms of detecting presence of the disease and would detect an animal that recovered after having been exposed to the disease 10 days or more.

### ***Programs That Would Support Rapid RVF Detection***

- Disease modeling and syndromic surveillance
- Electronic reporting system for animal abortions
- Definitive diagnosis of livestock abortions
- Sentinel animals or multiplex vector surveillance near ports of entry or international airports

The goal of such programs is to restrict an RVF event to a controlled area, unlike what occurred with WNV. Spraying is viewed as an effective strategy for controlling mosquito vectors in defined areas. Some have argued that had spraying commenced immediately after the identification of WNV, the disease would not have spread throughout the United States. It can be concluded that the immediacy of detection and effective vector control may well be the best measures to mitigate the spread of RVF.

### ***Wildlife Issues***

RVFV exhibits a very broad host profile, including many North American wildlife species. However, little is known about the role that the wildlife interface could play as a reservoir for the virus if it were to be introduced in the United States. Interestingly, researchers have been able to cultivate the virus in ticks with the virus remaining active for months. As such, it is possible that if RVFV were introduced in the United States, ticks could play a role in the relatively long-term maintenance of the virus, possibly implicating sequestered wildlife species.

## **3. Topic 2 Discussion Notes: Vaccines**

**Overview:** Presented by Dr. C. J. Peters (see [Appendix 9](#) for an outline).

### ***Human Vaccines Versus Animal Vaccines***

With zoonotic diseases there is a direct relationship between the threats to public health and those to animal health. Human and animal vaccines are a key disease control issue for zoonotic disease agents.

Efficacy is the primary concern for animal vaccines. However, for human vaccines, safety is the main concern. Veterinary science has the luxury of testing vaccines on the target animals and therefore is able to develop animal vaccines very quickly (partly because the associated clinical studies can be completed quickly). This has led to the development of a great number of successful veterinary vaccines. However, it has led the veterinary community to seek low-cost and low-technology vaccine solutions that are not supportive of an associated human vaccine program.

A vaccine strategy must be integrated into disease control and eradication programs. Very few control strategies for OIE List A diseases include the use of vaccines. The private sector and the research community need to be energized to deliver vaccines with the profile needed for control.

For a disease eradication program, wildlife vaccines must be considered. These have not been developed or tested, though this may become necessary. A delivery method such as food must be developed.

### ***Necessary Characteristics for New Vaccines***

They must

- Be marked to differentiate vaccinated animals from infected animals
- Have a rapid onset of immunity
- Provide an extended duration of immunity
- Prevent viremia so that RVFV cannot be amplified by the target host
- Meet standards for both human and veterinary use

### ***RVFV Vaccine Strategies***

If there is an event tomorrow, do we have a vaccine?

- For an immediate need, killed (formalin inactivated) vaccine may be the only option. It is stable and safe, but it requires three inoculations and a booster at 18 months. There are only about 10,000 primary vaccine courses, and the killed vaccine cannot be manufactured in the United States.
- For a near- or mid-term need, the MP-12 vaccine has demonstrated proof of concept with over 60 human inoculations. MP-12 appears safe and immunogenic; however, there are unresolved scientific issues and a need for a long-term sponsor. Capripox (recombinant) vaccine may be another candidate.

### ***OIE Standards for RVF***

If an RVF event were to occur in the United States, a great burden would be placed on the animal industry. The livelihood of millions of people would be jeopardized. After an initial RVF diagnosis, the period to attain the status of “RVF infection-free country or zone” is four years. Compared to OIE regulations for other foreign animal diseases, this is an exceedingly long timeframe. Even if the United States were able to obtain the status of “RVF-infected country/zone without disease,” for which the required period without an event is 6 months, numerous types of proof stating that the animals and humans are infection free would be required. It would take a large amount of resources (money, time, and people) to produce the necessary documents to reopen trade.

Smart identification approaches and methodologies will be critical. One other method that may aid the United States in reopening trade after a RVF event would be to use mosquito-free shipping containers. The difficulty with this approach is that there needs to be proof that the containers have *always* been mosquito free.

### ***Economic Impact Due to Trade Restrictions***

- Trade restrictions and requirements place a burden on producers. Producers would be required to follow numerous protocols for identifying animals that have or have not been infected, along with those that have been vaccinated.
- Trade partners react strongly to a foreign animal disease. Trade restrictions shut down livestock trade in Kenya when there was a RVF event.

## ANSER PROPRIETARY

Data herein subject to restrictions on the title page of this document.

- The United States follows OIE trade restrictions very closely; thus the United States can expect other countries to express the same desire in enacting trade restrictions when there is a foreign animal disease event within U.S. borders. A prime example of this is when the United States had an event of velogenic Newcastle disease (exotic Newcastle disease).
- It is possible that if the United States experiences an RVF event in one locality, be it as small as a single county in one state, the world will assume that the entire continental United States has been infected. However, through extensive testing, it may be possible to demonstrate that the disease is contained in one area or state and that animals and animal products coming from all other parts of the United States are safe.

### *Strategies Abroad*

- To be as prepared as possible, the United States needs to look beyond its borders and be aware of the virus activity in countries where the virus is endemic and where it is not.
- A complicating factor, which amplifies the danger of this disease, is that it is an arbovirus transmitted by mosquitoes, and mosquitoes can and do cross borders.

### *MP-12 Vaccine*

- Advantages
  - Development is near completion
  - Can be used on humans and animals (stockpiles could be shared)
- Drawbacks
  - MP-12 has not been used on very many humans. If there were an RVF event, the primary human population to be vaccinated would presumably be veterinarians.
  - Specific experiments have not been run to test whether MP-12 causes fetal loss. However, in other experiments, animals vaccinated with MP-12 have not experienced fetal loss.
- Key hurdles
  - Reversion to virulence
  - Marker (could be inserted, but testing may have to begin again)
- Issues to resolve
  - What is the current stock of MP-12?
  - What is the onset of immunity?
  - What is the duration of immunity?
  - What are the priorities for vaccination?
  - How fast can MP-12 be manufactured?
  - Where can MP-12 be manufactured?
  - What is the stock of reagent to monitor spread?

- Recognizing that there is no current market for MP-12 vaccine, there is a need to identify a product-partner manufacturer

### ***Capripox Vaccine***

- Advantages
  - Recombinant vaccine has advantages, including licensing issues
  - Vaccine is close to completion in Kenya
  - In developing the vaccine, a mild isolate of Capripox is used, then attenuated
- Drawbacks
  - The virus is the causative agent of lumpy skin disease, an OIE List A disease
  - Attenuated virus is grown in primary cell cultures

### ***Other Vaccine Candidates***

- Baculovirus (provides excellent protection in mice).
- Poxvirus vectors, including Capripox vaccines under development in Kenya.
- Genetically modified RVFV (“enhanced MP-12”).
- “Plum 3 strain”—has a gene deletion, so genetic reversion is not an issue.
- Alphavirus replicons—possibly the most promising for future development.
- Institut Pasteur vaccine. Note: There was no in-depth discussion concerning this vaccine candidate, as an Institut Pasteur representative could not be present for the WG meeting.

### ***Vaccine Production Issues***

- Licensing issues
- Need for master vaccine seed stock
- Need to test vaccine grown in different tissue culture cell lines
- Need for RVFV animal challenge testing
- Whether production can be done in the United States or must be done overseas
- Potential vaccine producers must be vetted

## **4. Topic 3 Discussion Notes: Epidemiology, Control, and Impact**

**Presentation:** Dr. K. J. Linthicum (see [Appendix 9](#) for outline).

**Presentation:** Dr. J. M. Wilson (see [Appendix 9](#) for outline).

The epidemiology of a mosquito-transmitted disease is very different than that of a directly communicable disease, such as FMD. Vector-borne diseases are especially difficult to predict and control, as evidenced by the continuing prominence in the tropics of malaria and dengue. The history of WNV is

particularly pertinent to us: from its focal appearance in New York in late 1999, it took only four years to spread across the continental United States.

Further complicating the epidemiology is that RVFV

- Is a zoonotic agent
- Infects a wide variety of mammals, including rodents, deer, and bats
- Can be transmitted by a number of American mosquito species
- Can be transmitted vertically from one mosquito generation to the next via eggs

“Stamping out” procedures, as employed against FMD, which rely on wholesale slaughter and stringent quarantine, are unlikely to be effective alone against a mosquito-borne disease. In fact, RVFV-infected cattle are infectious for only a few days.

Very few U.S. veterinarians have any experience with controlling vectored diseases; CDC is experienced, but joint USDA-HHS planning is not being done.

Central epidemiological questions after an RVF event is identified involve the best deployment of resources. These include

- How best to deploy surveillance
- What part mosquito control will play, who does it, and where
- How much vaccine is needed, and where it should be given
- How to deal with potential feral reservoirs (for example, white-tailed deer)?

Can an epidemic model be developed, possibly using remote sensing as a component, to help answer these questions on how to best marshal resources?

### ***Vector Control—Vaccine Balance***

Vaccine will directly and indirectly protect immunized cattle, as well as reduce the number of amplifying hosts such as humans.

Vaccine will not, however, remove the feral reservoirs or infected mosquito eggs, leaving the threat in place if vaccination is not continued. Vector control will play an important role.

- Vector control in the United States is locally supported; most counties do not have mosquito control apparatus. Who will perform control strategies—the military?
- Targeted spraying of livestock with insecticide to repel mosquitoes—this highly feasible approach is 90% effective.
- Experiments are needed to test effectiveness of different spraying strategies.

***Response Preparation***

APHIS should develop contingency plans with the CDC and practice these plans regularly.

Government and private clinicians should be fully educated on how to rapidly recognize RVF/RVFP. They also need to be knowledgeable as to the current testing methods and procedures needed to identify RVF/RVFP.

Programs to track types of information (such as abortions and weather) that can lead to pattern recognition must be developed. This will lead to prediction of the disease, which will then lead to rapid detection.

A database of baseline information on the rate of livestock abortions in the United States as well as other factors that could either foster or identify an RVF event (weather conditions, an increase in arthropod populations, etc.) needs to be created. Changes in the baseline can be easily observed, and the United States may be better prepared for a potential RVF event.

## **C. Working Group Findings, Key Concerns, Unresolved Questions, and Recommendations**

### **1. General Findings**

There are several reasons why RVF/RVFFV merits immediate attention:

- Introduction of RVFFV to the United States would severely disrupt the beef cattle and dairy industry and cause more significant human illness and likely mortality greater than WNV.
- RVFFV is a well-recognized potential biowarfare/bioterrorism agent. Furthermore, it has been steadily increasing its range of natural transmission from Africa to the Arabian peninsula.
- There exist critical gaps in our capability to detect, respond to, contain, and eradicate RVFFV and RVF.
- Regardless of how RVFFV may be introduced, the nature of its vector-borne epidemiology means that if it is not rapidly contained, it may spread as rapidly as WNV, but with far more serious consequences.
- Decision makers are generally unaware of the significant threat the virus and disease pose to the United States.

### **2. RVFFV Detection and Identification**

#### *Findings*

- Past RVF epizootics have been recognized because of RVF first being detected in the human community.
- The most likely tip-off event for an RVFFV infection would be an animal abortion storm in a region with favorable climatology for the principal vector. Currently, over 50% of livestock abortions are never definitively diagnosed. A comprehensive abortion-testing program, with an appropriate RVFFV testing protocol, offers the possibility of detecting the first case.
- Field veterinarians and investigators will need to send suspect samples to an accredited laboratory for testing. These diagnostic tools and testing protocols must be validated. Policies and procedures for their use must be established. Following development of these policies and procedures, the systems should be made fully operational and included in the standard suite of tools for state and regional laboratories and, in time, at numerous other locations across the United States, as fitting.
- There is no one single method (such as RT-PCR) for detecting and identifying RVFFV. A supporting laboratory program must have the ability to use classical approaches (virus isolation and serology), pathology methods (specific immunohistochemistry), and modern molecular diagnostic techniques.
- Other detection strategies for RVFFV include establishing electronic reporting networks for animal abortions, the use of disease and environmental modeling, and expanding the existing multiplex vector-sampling programs. A National Biosurveillance Integration System is in development at DHS and is designed to provide situational awareness to detect events earlier by combining data from the CDC, USDA, and HHS with environmental monitoring from BioWatch and comparing it with threat and intelligence information.

- RVFV detection in the human populations faces the same problem as in animals: physicians are not predisposed to look for the virus. An investigation of RVFV would not likely occur until the presence of acute symptoms in a large number of humans was observed.
- No studies have been done on the susceptibility of various species of North American wildlife to RVFV or how best to control or treat an event within the wildlife population.

### ***Key Concerns and Unresolved Questions***

- There is no established and comprehensive nationwide veterinary and public health awareness and training program dealing with RVF that is fully available to all levels of health practitioners.
  - How should this large-scale training be conducted?
  - How broad should the target audience be?
  - How long will it take to achieve the requisite level of training and awareness?
- Only limited testing and identification tools are available.
  - What type and what quantity are needed to address RVFV?
  - How long will it take to validate the testing methods and procure needed for test kits?
  - Where should these testing capabilities be located?
- An appropriate abortion-testing protocol has not been determined.
- Current multiplex vector testing and surveillance do not include RVFV.
  - Which testing programs are suitable candidates for the addition of RVFV testing?
  - What steps must be taken to add RVFV?
  - Does wildlife serve as RVFV amplifiers?

### ***Recommendations***

- Conduct appropriate large-scale veterinary and public health training and awareness for RVF
- Improve the reporting and investigation of animal abortions
- Develop disease models for RVF
- Validate laboratory-testing protocols for the presence of RVFV
- Add RVFV to appropriate existing multiplex disease vector sampling programs

## **3. RVF Vaccines**

### ***Key Findings***

- There is no licensed commercial vaccine (that meets U.S. standards of safety and efficacy) available for RVF. In the United States, the only current vaccine consists of an unknown stock of an experimental MP-12 vaccine. While MP-12 has demonstrated a proof of concept in limited testing, there are scientific issues that must be resolved, and additional studies will be required to do so.

Following the initiation of dedicated effort, the resolution of these issues could take from 12 to 24 months to accomplish.

- The development of suitable candidate RVF vaccines within the United States will require a considerable investment from both the Federal Government and the private sector. Also, for optimal development, the effective use of cutting-edge technologies will need to be employed. Since these technologies are expensive, industry cannot justify the investment without a suitable market for its product. In the United States, foreign animal disease vaccines have no commercial market until a disease is introduced. In the case of RVF, it will be critical for the Federal Government to form alliances with private-sector vaccine manufacturers to produce and stockpile sufficient quantities of vaccines.
- If an RVF event were to occur today in the United States, a killed (formalin inactivated) vaccine could be used. However, there is no significant stock of this vaccine in the United States. Additionally, the amount of stock available outside the United States has not been determined. This type of vaccine cannot be produced in the United States, since it initially requires the use of a live virus and would therefore subject the United States to OIE trade restrictions. In consideration of the immediate need for an RVF vaccine, a top priority is to find an overseas industry partner that can meet the requirements to handle and produce a safe and efficacious killed RVF vaccine for use in the United States.
- In Kenya, there is ongoing development and testing on a live attenuated recombinant Capripox vaccine. Although there are important issues regarding this vaccine, under certain circumstances it may be suitable for emergency use in the United States. However, the supply of this vaccine and the rate of production are unknown. Furthermore, the vaccine cannot be manufactured in the United States (live Capripox vaccines are not permitted in the United States, as this virus is the causative agent of lumpy skin disease, an OIE List A disease).

### ***Key Concerns and Unresolved Questions***

- The stockpile of RVF killed vaccine that is suitable and immediately available for emergency use in the United States has not been determined.
  - How many doses of the vaccine can the United States obtain in an emergency?
  - How many injections are required for vaccination, and over what period of time?
  - What is known regarding the onset of immunity and the duration of immunity?
  - How many doses will be required for laboratory workers and first responders?
  - What are the options for industry partnership outside the United States?
  - How many doses should be maintained on hand for emergency use?
- Development of the MP-12 vaccine will take 12-24 months.
  - What amount of current stock of MP-12 is available?
  - How fast can MP-12 be produced, and where can it be manufactured?
  - How many doses are needed to stockpile in sufficient quantities to deal with an RVF event in the United States?

### ***Recommendations***

- Establish the requirements for an emergency U.S. RVF vaccination program. This effort should include a partner relationship with one or more international vaccine manufacturers.
- Investigate obstacles to full development of the MP-12 vaccine. Concurrently, evaluate the merits of alternative candidate vaccines (including killed vaccine, Clone 13, and the Kenyan Capripox). Once a candidate vaccine has been selected, then development, production, and stockpiling should proceed quickly. Additionally, plans for the distribution, and policies for the use, of this vaccine must be developed and executed.

## **4. RVF Epidemiology, Control, and Impact**

### ***Key Findings***

- APHIS does not have an approved response plan with specific written procedures to effectively deal with RVFV or other vector-borne pathogens. Additionally, to be effective against zoonotic agents, such a plan must be coordinated with the CDC, other public health elements, and numerous other responsible Federal and state departments and agencies.
- Mosquito-borne diseases are much more difficult to control than directly communicable diseases because of the unpredictability of transmission and because, by passing the virus through their ova, mosquito vectors act as viral reservoirs.
- Vector control will play a major part in epidemic suppression. Unfortunately, most cattle production states have no local mosquito control organizations.
- Modeling and remote sensing can be used to design plans for the most effective vaccine and mosquito control response.
- There is no paradigm on how to most efficiently vaccinate, quarantine, or control mosquitoes around discovered foci.

### ***Recommendations***

- APHIS and CDC should develop an RVF response strategy and practice these plans regularly.
- ARS and APHIS should develop contingency models to guide effective control measures.
- Establish a network of veterinary clinician and vector control first responders.
- Test innovative methods for containment, such as insecticide treatment of cattle.

## D. Consolidated and Prioritized Recommendations

The following is a consolidated and prioritized list of recommendations derived from the RVF WG meeting:

1. Develop a joint APHIS/CDC RVF cooperative response strategy and associated contingency plan.
2. Establish the requirements, plans, procedures, and protocols for an emergency RVF vaccination program for the United States. This effort should include a partnership with one or more international vaccine manufacturers.
3. Conduct appropriate veterinary and public health training and awareness for RVF through USDA/CDC interagency cooperative arrangements.
4. Improve the reporting and investigation of animal abortions, possibly utilizing already implemented human syndromic surveillance systems.
5. Validate laboratory diagnostic tools and testing protocols for RVF/RVFP and establish policies and procedures for their use. Following the development of these policies and procedures, the systems should be made fully operational in a variety of venues.
6. Overcome obstacles to full development of promising animal and human candidate vaccines. A primary safe and efficacious vaccine should be selected. To do so, further research, development, and evaluation of the MP-12 RVF vaccine, as well as promising alternative candidate vaccines, should be fully supported.
7. Once the best candidate RVF vaccine has been selected for full development, production and stockpiling should proceed rapidly. Concomitantly, plans for the distribution, and policies for the use, of this vaccine must be firmly established.
8. A wide range of epidemiological disease models for RVF/RVFP needs to be developed, tested, and validated. These include the following:
  - Epidemic triggering at foreign epidemic sites
  - Tracking translocation through the air transportation grid to introduction stateside
  - Local epidemic triggering, propagation, and ecological establishment
9. RVF control strategies should be thoroughly tested and evaluated.
10. Add RVFP to appropriate existing multiplex vector sampling programs.
11. Use interagency cooperation, for example, between USDA and CDC, to explore threat reduction through international partnerships with countries where RVFP is endemic (such as Kenya and Egypt).

## Appendix 1. August 2004 RVF Working Group Participants

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## Appendix 2. General Characteristics of RVF and Zoonotic Diseases<sup>4</sup>

**1. Pathogen threats to animal health.**<sup>5</sup> Infectious diseases that pose an international threat to animal and human health are considered potential biological threat agents.

The authoritative international organization for animal health is OIE, better known as the World Organization for Animal Health. The OIE lists RVF among 15 List A diseases ([Appendix 4](#)) based on their high morbidity and mortality, their potential for rapid spread, serious economic and/or public health consequences, and impact on the international trade of animals and animal products. OIE member countries are required to immediately report an event involving any infectious agent on this list.

The Public Health Security and Bioterrorism Preparedness and Response Act required the Secretary of Agriculture to establish a list of select biological agents that have the potential to pose a severe threat to animal and plant health. This responsibility was assigned to APHIS, which classified 20 microorganisms or toxins that pose a risk to *both* human and animal health as “overlap agents or toxins.” Unlike WNV, RVFV is included on both this list ([Appendix 5](#)) and the OIE List A.

**2. Potential impact of biological threat agents.**<sup>6</sup> The criteria for classifying biological threat agents must take into account the multidimensional array of direct and hidden costs to the Nation if we were to experience an intentional or unintentional event. As an example, the 1986 event of bovine spongiform encephalopathy in the United Kingdom was reported to have cost the European Union as a whole over \$100 billion.

The severity of impact that a biological threat agent can have on agriculture may depend on whether the agent is foreign, emerging, or domestic. Of particular concern are animal pathogens that can spread disease to epizootic proportions, those that have complex life cycles with insect and wildlife reservoirs, or those that can be weaponized for biological warfare. In the United States, the zoonotic potential of an agent should be given extra weight, since the impact of an event on public health and the loss of confidence in our food supply could have a significant impact on our Nation. Such an impact includes not only the potential loss and cost associated with animal and human disease, but also the second- and third-order effects on the agricultural and public health industries, as well as the economy at large (for example, livestock feed suppliers, health care insurance, and the food-service industry). In addition, the OIE imposes a trade ban of four years on countries with endemic RVF, conditionally allowing trade after six months in an event-free status. For 2003, the United States had over \$5.7 billion of beef-related exports alone.

The interagency report “Strategic Research Targets to Protect American Livestock and Poultry from Biological Threat Agents” identified 10 animal pathogens from USDA’s list of select and overlap agents

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<sup>4</sup> Except as noted, this background section draws heavily from the body of “Strategic Research Targets to Protect American Livestock and Poultry from Biological Threat Agents,” report from the WMD Counter Measures Working Group, Animal Pathogen Research and Development Subgroup, 31 October 2003.

<sup>5</sup> *Ibid.*, p. 4.

<sup>6</sup> *Ibid.*

that pose great harm to the Nation if an event were to occur. RVFV is one of those select agents ([Appendix 6](#)). [Appendix 7](#) summarizes the status of animal vaccines for the 10 threat agents; [Appendix 8](#) summarizes, rank-orders, and prioritizes research needs, identifying RVF as the number 3 priority (behind FMD and highly pathogenic avian influenza). In spite of the fact that RVFV is identified as a biological threat agent, is considered one of USDA's "select and overlap agents," is ranked as the number three biological threat agent in order of research priority, and presents a broader impact in terms of animal and human morbidity and mortality than WNV, there is no animal or human vaccine commercially available that meets U.S. standards for safety and efficacy.

**3. Vaccine profile.**<sup>7</sup> There is consensus among animal health officials that in a disease event, vaccination rather than depopulation is the most cost-effective strategy. However, several concerns about the safety and efficacy of vaccines on the international market today limit their use in controlling diseases. Safety issues commonly include concerns over contamination with extraneous agents, inadequate inactivation of killed vaccines, the potential of some vaccinated animals to continue shedding pathogenic virus, reversion to virulence of modified live vaccine strains, the potential negative effects of adjuvant systems on the production of food animals, and adverse systemic immunological reactions. Efficacy issues are just as complex but revolve around the manner in which a vaccine is formulated and quality controlled during production, as well as the clinical parameters used to determine efficacy. An additional key concern is the discrepancy in the performance of vaccines under experimental control conditions versus their actual performance in the field.

The development of vaccines for biological threat agents is particularly challenging. Not only do these vaccines need to prevent clinical disease, but they also need to present an efficacy profile that enables control and eradication strategies without interfering with the ability of a country to obtain and maintain OIE infection-free status. Therefore, vaccines for biological threat agents must be designed to prevent or significantly reduce shedding of the etiological agent and spread of infection. The vaccine must be marked so that infected animals can be differentiated from vaccinated animals.

**4. Manufacturing and private industry.**<sup>8</sup> Vaccine development for diseases that are regulated because of their impact on trade presents a particular challenge for private industry, since the sales for these products (and their companion diagnostic tests) are totally dependent on Government sponsorship of a vaccine program. Therefore, industry cannot rely on traditional business models to determine sales projections and profits, which are critical to business plans and the decision to support future research and development initiatives. Furthermore, the development of highly efficacious vaccines that have the profile to control and eradicate animal pathogens requires an investment in cutting-edge technologies. Since such new technologies are expensive, industry cannot justify the investment if there is only a small market for their product. In addition, vaccines for foreign animal diseases in the United States have no market until a disease is introduced.

In addition, the cost of production may be prohibitive if the manufacture of the vaccine requires dedicated production runs or if the vaccine cannot be integrated into high-throughput manufacturing facilities. Contract manufacturers with state-of-the-art production facilities in the United States may need to be considered to ensure the containment of biological threat agents during production. Manufacturers need to be scrutinized for their ability to prevent the contamination of their product with extraneous agents.

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<sup>7</sup> Ibid. p. 5.

<sup>8</sup> Ibid. p. 6.

Regulatory requirements are not uniform across countries; therefore, consideration should be given to good manufacturing practices. Requirements that define good manufacturing practices include where the product will be manufactured and an assessment of the cost against the level of quality assurance needed to ensure that the final product is safe, pure, potent, and efficacious.

Overcoming the challenges of regulation, technology, and production is no guarantee that a vaccine will be used or that contracts to manufacture it will be forthcoming. The formation of key alliances between the Government and the private sector, through formal contractual agreements, may be necessary to mitigate the risks that biological threat agents pose to our Nation.

**5. RVF vaccines.**<sup>9</sup> In areas of Africa where the virus is endemic and during RVF events, a live vaccine prepared from the Smithburn attenuated strain of RVFV has been used as a control in nonpregnant cattle and sheep. In RVF-free countries within Africa, an inactivated vaccine (prepared from virulent field strains) has been used in pregnant animals. The inactivated vaccine is carefully tested to ensure that there is no residual live virus.

For humans, an inactivated experimental RVFV vaccine has been used for 25 years with considerable success to protect field and laboratory personnel. This vaccine is produced on diploid cells; however, the limited availability of the vaccine precludes its use in the general population.

Two new experimental vaccine candidates, produced from human RVFV isolates, are undergoing extensive testing with a view to replacing existing vaccines. The first, MP-12, is a mutagen-derived strain of virus. Immunogenicity and pathogenicity have been tested in sheep, and the virus was found to be non-abortionogenic in pregnant ewes. MP-12 was found to be protective in young lambs and in cattle. Further testing in sheep demonstrated that the use of the vaccine after 28 days of pregnancy resulted in abortions and severe fetal teratology.

The second candidate, Clone 13, was found to be avirulent in mice and hamsters and highly immunogenic. Immunogenicity and pathogenicity have been tested in lambs, sheep, and young and adult goats. In further trials, it was non-abortionogenic in pregnant sheep and gave more than 80% protection from virulent challenge. Clone 13 is marked by a large deleted portion of the sRNA segment coding for the nonstructural proteins, which should result in a stable vaccine candidate.

**6. Clinical features of RVF in animals.**<sup>10</sup> The major clinical features of RVF in animals are outlined in Table 2. Signs in affected animals range from peracute to inapparent, and susceptibility depends on breed, genotype, and age.

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<sup>9</sup> "Manual of Diagnostic Tests and Vaccines for Terrestrial Animals," OIE, updated 23 July 2004, Section 2.1.8. Available at [http://www.oie.int/eng/normes/mmanual/A\\_index.htm](http://www.oie.int/eng/normes/mmanual/A_index.htm).

<sup>10</sup> Center for Infectious Disease Research and Policy, University of Minnesota, "Viral Hemorrhagic Fever (VHF): Current, Comprehensive Information on Pathogenesis, Microbiology, Epidemiology, Diagnosis, Treatment, and Prophylaxis," last updated 31 August 2004. Available at <http://www.cidrap.umn.edu/cidrap/content/bt/vhf/biofacts/vhffactsheet.html>.

| Feature                | Characteristics   |
|------------------------|---|
| <b>Cattle</b>          |   |
| Incubation period      | 1-6 days  |
| Clinical signs         | Calves: fever of 40°-42°C (104°-106°F); depression; icterus; anorexia and weakness; listlessness; evident abdominal pain<br>Adults: fever of 40°-42°C (104°-106°F); excessive salivation; anorexia; weakness; fetid diarrhea; fall in milk yield; nasal discharge |
| Case-fatality rate     | Calves: 10%-70%; adults: <10%   |
| <b>Sheep and Goats</b> |   |
| Incubation period      | Lambs: 12-36 hrs<br>Adults: 1-6 days  |
| Clinical signs         | Lambs: fever of 40°-42°C (104°-107°F); anorexia and weakness; listlessness; evident abdominal pain<br>Adults: fever of 40°-41°C (104°-106°F); mucopurulent nasal discharge; vomiting; anorexia; listlessness; diarrhea; icterus                                   |
| Complications          | Abortion rates can reach 100% (aborted fetus often autolysed); peracute hepatic disease in lambs and kids <1 wk of age; hepatitis; cerebral infections; ocular infections   |
| Case-fatality rate     | Lambs <1 wk of age, as high as 100%; >1 wk of age, as high as 20%; adults 20%-30%   |

**Table 2—Clinical Features in Animals**

**7. Public health issues and clinical features of RVF in humans.**<sup>11</sup> During epizootics in animal populations, infection can spread to humans and result in concurrent epidemics of human disease. Human infection can occur through several mechanisms: bites from infected mosquitoes or other vectors, direct contact with infected animal tissues, and aerosols generated during slaughter or in the laboratory setting.

Human clinical signs are more severe than those of animals, and age offers no protection from severe illness. Distinct characteristics of RVFV infection in humans are outlined in [Table 3](#).

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<sup>11</sup> Ibid.

| Features                | Characteristic  |
|-------------------------|---|
| Incubation period       | 2-6 days  |
| Prodrome                | Fever, headache, photophobia, retro-orbital pain  |
| Clinical signs/symptoms | Subclinical infection common; four clinical patterns: undifferentiated fever lasting 2-7 days (>90% of cases, often associated with nausea, vomiting, and abdominal pain); hemorrhagic fever with marked hepatitis and bleeding manifestations (<1% of cases; occurs 2-4 days after onset of fever); encephalitis (<1% of cases; occurs 1-4 wks after onset of fever); retinitis (up to 10% of cases; occurs 1-4 wks after onset of fever; often bilateral; hemorrhages, exudates, and cotton wool spots may be visible on macula; retinal detachment may occur); common bleeding manifestations include gastrointestinal bleeding and epistaxis; neurologic symptoms include confusion, lethargy, tremors, ataxia, coma, seizures, meningismus, vertigo, choreiform movements; hepatitis, hepatic failure, and renal failure may occur; a report of the 2001 event in Saudi Arabia identified the following clinical features for 683 laboratory-confirmed cases: fever, 92.6%; nausea, 59.4%; vomiting, 52.6%; abdominal pain, 38.0% diarrhea, 22.1%; jaundice, 18.1%; neurologic manifestations, 17.1%; hemorrhagic manifestations, 7.1% |
| Features                | Characteristic  |
| Laboratory features     | Initial leukocytosis may occur, followed by leukopenia; thrombocytopenia in severe cases; laboratory features of DIC in severe cases (prolonged bleeding time, prothrombin time, and activated partial thromboplastin time; elevated fibrin degradation products; decreased fibrinogen); elevated hepatic enzymes (such as alanine aminotransferase and aspartate aminotransferase) and bilirubin   |
| Complications           | Blindness following retinitis; neurologic sequelae following encephalitis   |
| Case-fatality rate      | In the 2000 event in Saudi Arabia, case-fatality rate was reported as 13.9% among symptomatic patients; death usually due to hepatic necrosis and disseminated intravascular coagulation  |

**Table 3. Clinical Features in Humans**

**8. Differential diagnosis.**<sup>12</sup> Few veterinarians are trained to recognize exotic animal diseases. RVF can be mistaken for many endemic viral diseases and may consequently become firmly established prior to its recognition. RVF does manifest itself in a distinct manner and can be identified by these characteristics:

- Sudden onset of many livestock abortions at all stages of pregnancy; these may affect a restricted area or be countrywide
- Acute febrile disease with high fatality rates in young animals
- Liver lesions present in all cases
- Associated with high mosquito populations and/or flooding of grasslands
- May be associated with an influenza-like disease in humans

**9. Laboratory diagnosis.**<sup>13</sup> RVFV presents a serious hazard to all personnel handling infected carcasses, blood, and other tissues both in the field and in the laboratory. For this reason, the diagnostic testing

<sup>12</sup> *Recognizing Rift Valley Fever*, F. Glyn Davies, Food and Agriculture Organization of the United Nations, Rome 2003. Available at [http://www.fao.org/documents/show\\_cdr.asp?url\\_file=/docrep/006/y4611e/y4611e00.htm](http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/006/y4611e/y4611e00.htm).

<sup>13</sup> Ibid.

approach that is chosen will depend on the facilities available that can be safely used to contain such a highly pathogenic disease. There are two types of test: the first identifies or isolates the RVFV or antigen; the second demonstrates the presence of rising titers of the RVFV-specific antibody or IgM. The clinical diagnosis can be confirmed by a number of tests that include

- Histopathological examination of the liver of affected animals.
- Virus isolation from serum and blood collected during the febrile stage of the disease from liver, spleen, and brain of animals that have died or from aborted fetuses. Primary isolation is usually performed in hamsters, in infant or adult mice, or on cell cultures of various types.
- Detection of viral antigen by immunofluorescent or immunoperoxidase staining of frozen sections, immunodiffusion, complement fixation, and ELISA.
- Detection of viral RNA by RT-PCR.
- Detection of antibodies by virus neutralization and ELISA (not the hemagglutination-inhibition test, which is non-specific); these are used mainly retrospectively to determine the extent of an epidemic. For diagnostic confirmation, recent or current infection must be distinguished from pre-existing immunity. Paired samples collected during the acute phase and again 2 to 3 weeks later provide evidence of recent infection. IgM-capture ELISA allows diagnosis of recent infection to be made on a single serum sample.

**10. Hemorrhagic fever viruses as biological weapons.**<sup>14</sup> Animal studies on primates have demonstrated that clinical infection can be caused by aerosolized preparations of a number of hemorrhagic fever viruses, including the Ebola, Marburg, Lassa, and yellow fever viruses. Additional viruses (including RVFV) have been shown to cause aerosol infections in the laboratory setting. These viruses are considered potentially suitable as biological weapons because

- They can be disseminated through aerosols
- They have a low infectious dose
- They cause high morbidity and mortality
- They cause fear and panic in the general public
- Effective vaccines are not available or supplies are limited
- These pathogens are available and most can be readily produced in large quantities

Research on weaponizing various hemorrhagic fever viruses has been conducted in the past despite the lack of treatment options or protective vaccines. RVFV was developed as a biological weapon by the U.S. offensive weapons program prior to its termination in 1969.<sup>15</sup>

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<sup>14</sup> “Viral Hemorrhagic Fever (VHF): Current, Comprehensive Information on Pathogenesis, Microbiology, Epidemiology, Diagnosis, Treatment, and Prophylaxis,” University of Minnesota Center for Infectious Disease Research and Policy, last updated 31 August 2004. Available at <http://www.cidrap.umn.edu/cidrap/content/bt/vhf/biofacts/vhffactsheet.html#Bioweapons>.

<sup>15</sup> “Hemorrhagic Fever Viruses as Biological Weapons,” *Journal of the American Medical Association*, vol. 287, no. 18, 8 May 2002.

## Appendix 3. Illustrative RVF Event Scenario

**Prologue—March 2005.** A group of foreign operatives has been anonymously living in the United States for several years. A key member, who happens to be a trained veterinarian, makes a routine visit to one of the group's locations in Pakistan. As the prime mover for an attack on livestock, he is pleased that the leadership has accepted his ideas. He now receives the blessing to make use of his two-year survey of the U.S. livestock industry's most vulnerable stockyards (located in California, Louisiana, and New York) and finalizes his recruitment of surrogate individuals with a sustained track record of work at these sites. He returns to the United States to test his surrogates' ability to covertly spray a harmless test water mist over animals at each location. Following several months through the early spring, he determines that the surrogates are sufficiently trustworthy, hooked on the pay, and fully assured that their actions, although a bit odd, are harmless. Luck is on his side, as this is a good year when the natural convergence of seasonality and heavy rainfall in the United States are optimal for an attack. He then boards a flight from the United States to the Middle East, where his organization's secure biological laboratory is located. He returns within days, carrying a shaving kit with cologne and mouthwash bottles, each holding 0.5 liter of centrifuged supernatant containing high concentrations of RVFV derived from Vero cell cultures.

**The attacks.** Directly upon his return, the viral suspensions are divided into several aliquots innocuously transported by air to California, Louisiana, and New York. The surrogates are provided with the usual water bottles and, within 24 hours, believing it is business as usual, they successfully disperse an aerosol mist containing RVFV within the stockyards. The following number of animals and humans are infected with RVFV. This is Day 1:

- California: 537 cows and sheep, and 29 human stockyard workers
- Louisiana: 652 beef cattle and 23 human stockyard workers
- New York: 403 cows and 19 human stockyard workers

As a result of normal commerce at the stockyards, within hours a large percentage of the infected animals are shipped to multiple locations both in and out of state.

**The initial events.** The infected animals rapidly develop a significant viremia. Within five days, mosquitoes are feeding on the infected animals, and at this point RVFV is effectively introduced into the local mosquito populations throughout California, Georgia, Louisiana, New York, and Pennsylvania. These mosquitoes in turn begin to infect local animal and human populations at each location. (By Day 27, the number of infected animals and humans will have tripled).

By Day 6, the infected livestock begin to exhibit the initial symptoms of RVF. The majority of the animals run fevers and become lethargic. Those sheep that were pregnant abort within days, and nearly all of the pregnant dairy cows abort within seven weeks.

By Day 13, more than 20% of the young lambs and calves die, as well as 15% of the adult sheep and cattle. Surviving animals serve as amplifiers and reservoirs for RVFV, allowing the virus to become firmly established in the local mosquito population and spread to the surrounding areas. In some cases, the animal symptoms are ignored as common illnesses. In others, the symptoms are misdiagnosed and the animals are treated with antibiotics. Test results repeatedly come up inconclusive. Unknown to U.S. authorities, at this point RVF is now an endemic disease in five states. (As a result of the geographic

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dispersion of the infected animals, errors in diagnosis, and the length of time to complete various supporting tests, RVF is not identified until Day 27).

In the case of the 71 humans initially infected, by Day 7, the majority begins to show flu-like symptoms consistent with RVF (low-grade fever, malaise, and fatigue). In most cases the symptoms are treated with over-the-counter medications and disappear within a week. Due to the geographic dispersion of the infected persons, hospitals and health care workers are initially unable to recognize the pattern of infection. After four weeks, nine of the persons infected by the initial attack have developed severe symptoms, including retinitis, encephalitis, tremors, seizures, and renal failure. Six of these die.

By Day 14, 17 farmers have been infected as a result of close contact with animal carcasses, blood, and body fluids. In addition, 57 employees at a meat-processing plant in Georgia become infected through close contact with infected carcasses and aerosols generated by the meat-cutting and -processing machinery. Of these 74 cases, two will die within a month from complications of encephalitis, initially attributed to a local event involving WNV. Four others will develop severe symptoms and eventually die.

By Day 21, the health departments in California, Georgia, Louisiana, New York, and Pennsylvania begin to recognize a rise in the number of individuals with apparent cases of flu; however, the patients are treated for their symptoms, and there is no suspected connection between the human flu cases and the growing animal illnesses.

By Day 25, reports of spontaneous animal abortions reach the offices of state veterinarians in California, New York, and Pennsylvania. In Louisiana, following necropsy of a number of dead animals, a veterinary pathologist determines that the livers all exhibit similar abnormalities. He contacts the state veterinarian, who calls USDA’s APHIS. APHIS then directs that the samples be securely transported to Plum Island Animal Disease Center using strict auditing and biohazard containment procedures. Within two days, following growth of the virus in appropriate tissue culture cell lines, the APHIS staff at Plum Island supplies the results to their Administrator, who passes the findings to the Secretary of Agriculture. At this point, the Secretary announces publicly that USDA has made an initial diagnosis of RVF in the United States.

*Status of the Event as of Day 27*

| State        | Livestock    |            | Human      |          |
|--------------|--------------|------------|------------|----------|
|              | Infected     | Dead       | Infected   | Dead     |
| California   | 1,594        | 214        | 87         | 2        |
| Georgia      | 152          | 9          | 72         | 2        |
| Louisiana    | 1,813        | 134        | 69         | 2        |
| New York     | 1,007        | 89         | 57         | 2        |
| Pennsylvania | 207          | 15         | 11         | 1        |
| <b>Total</b> | <b>4,773</b> | <b>461</b> | <b>296</b> | <b>9</b> |

Immediately following the diagnosis for RVF, a WG is convened to develop an emergency response plan. This group consists of appropriate representatives of USDA, HHS, and selected members of other Federal

elements, as well as representatives from the affected and bordering states. Some key issues they must consider include

- Generating a rapid and effective public awareness program
- Determining the availability and location of vaccine worldwide
- Developing virus surveillance, control, and eradication strategies
- Employing or rapidly developing accurate models of disease spread
- Defining and identifying high-risk areas and populations
- Initiating and, if need be, developing a diagnostic testing protocol
- Determining the specific actions needed to accomplish effective quarantine and/or limits to livestock movement
- Identifying the origin of the RVF introduction
- Preparing to provide technical and other support for indemnification determinations

**Epilogue.** By Day 100, the event has been contained, largely as a result of seasonal temperature change and the resulting impact on the mosquito vector. More than 12,000 animals have been infected, and 1,029 humans are known to have been infected. By the end of the year, 114 human deaths are attributed to the attack, and individuals with permanent disease-related disability number in the hundreds.

In April of the following year, the first mosquitoes begin to hatch in Louisiana. Many of the larvae carry the RVFV as a result of transovarial transmission. By the end of 2006, RVF is considered endemic in 29 states. At the end of the following year, it is endemic to all 48 contiguous states. The economic impact to livestock and related industries, public health, trade, and tourism is estimated at greater than \$50 billion.

## **Appendix 4. Office of International Epizootics “List A” Diseases<sup>16</sup>**

Foot-and-mouth disease

Swine vesicular disease

Peste des petits ruminants

Lumpy skin disease

Bluetongue

African horse sickness

Classical swine fever

Newcastle disease

Vesicular stomatitis

Rinderpest

Contagious bovine pleuropneumonia

Rift Valley fever

Sheep pox and goat pox

African swine fever

Highly pathogenic avian influenza

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<sup>16</sup> “Strategic Research Targets to Protect American Livestock and Poultry from Biological Threat Agents,” p. 30.

## Appendix 5. Overlap Agents<sup>17</sup>

*Bacillus anthracis*

Botulinum neurotoxins types A-G (0.5 mg)

Botulinum neurotoxin producing species of

*Clostridium* (0.5 mg)

*Brucella abortus*

*Brucella melitensis*

*Brucella suis*

*Burkholderia mallei*

*Burkholderia pseudomallei*

*Clostridium botulinum*

*Clostridium perfringens epsilon toxin* (100 mg)

*Coccidioides immitis*

*Coxiella burnetii*

Eastern equine encephalitis virus

*Francisella tularensis*

Hendra virus

Nipah virus

Rift Valley fever virus

Shigatoxin (100 mg)

T-2 toxin (1,000 mg)

Venezuelan equine encephalitis

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<sup>17</sup> Ibid.

## Appendix 6. Select Biological Threat Agents<sup>18</sup>

| Agent  | Classification   | Host Range  | Reservoir                                       | Distribution   |
|--|--|---|---|--|
| Foot-mouth-disease virus   | Family <i>Picornaviridae</i><br>Genus <i>Aphthovirus</i><br>Seven serotypes  | Wild and domestic cloven-footed animals                   | Domestic livestock and wildlife                 | Asia<br>Africa<br>South America                        |
| Avian influenza virus  | Family <i>Orthomyxoviridae</i><br>Genus <i>Influenzavirus A</i><br>15 hemagglutinin subtypes<br>9 neuraminidase subtypes | Avian species<br>Zoonotic <sup>2</sup>                    | Wild migratory waterfowl<br>Sea and shore birds | Worldwide  |
| Newcastle disease virus  | Family <i>Paramyxoviridae</i><br>Genus <i>Rubulavirus</i>  | Avian species   | Domestic poultry                                | Worldwide  |
| Rift Valley fever virus  | Family <i>Bunyaviridae</i><br>Genus <i>Phlebovirus</i><br>Arbovirus <sup>1</sup> (mosquitoes)                            | Cattle, sheep, goats<br>Zoonotic<br>Sylvatic <sup>3</sup> | Sheep and cattle                                | Africa   |
| Nipah & Hendra viruses   | Family <i>Paramyxoviridae</i><br>Genus <i>Henipavirus</i>  | Pigs<br>Zoonotic<br>Sylvatic                              | Fruit bats                                      | Indian subcontinent<br>Southeast Asia<br>Australia     |
| Classical swine fever virus  | Family <i>Flaviviridae</i><br>Genus <i>Pestivirus</i><br>One serotype  | Pigs<br>Wild boar   | Pigs  | EU<br>Africa<br>South America<br>Asia                  |
| Venezuelan equine encephalitis virus   | Family <i>Togaviridae</i><br>Genus <i>Alphavirus</i><br>Arbovirus (mosquitoes)   | Horses<br>Zoonotic<br>Sylvatic                            | Horses  | South America  |
| African swine fever virus  | Family <i>Asfarviridae</i><br>DNA arbovirus (ticks)<br>(similar to pox virus)  | Pigs  | Wild pigs                                       | Africa   |
| Rinderpest virus   | Family <i>Paramyxoviridae</i><br>Genus <i>Morbillivirus</i><br>One serotype  | Wild and domestic cloven-footed animals                   | Wild ungulates <sup>4</sup>                     | Indian subcontinent<br>Near East<br>Sub-Saharan Africa |
| Eastern equine encephalitis virus  | Family <i>Togaviridae</i><br>Genus <i>alphavirus</i><br>Arbovirus (mosquitoes)   | Horses<br>Zoonotic<br>Wild birds<br>Sylvatic              | Wild birds                                      |  |
| <sup>1</sup> Arbovirus: insects are infected by the virus and transmit the virus to animals and/or humans<br><sup>2</sup> Zoonotic: causes disease in humans<br><sup>3</sup> Sylvatic: infects and cycles in wild-life<br><sup>4</sup> Ungulates: any hoofed quadruped |  |   |   |  |

<sup>18</sup> “Strategic Research Targets to Protect American Livestock and Poultry from Biological Threat Agents,” p. 33.

## Appendix 7. Animal Vaccines for Biological Threat Agents<sup>19</sup>

| Agent                                | Trade Issues <sup>1</sup> | Vaccine/Efficacy <sup>2</sup> | Duration <sup>3</sup> | Onset <sup>4</sup> | Pen-Side Test <sup>5</sup> | Stockpile <sup>6</sup> |
|--------------------------------------|---------------------------|-------------------------------|-----------------------|--------------------|----------------------------|------------------------|
| Foot & mouth disease virus           | Yes                       | Yes/medium                    | 6 months              | 2 weeks            | No                         | Yes                    |
| Avian Influenza virus                | Yes                       | Yes/medium                    | 5 months              | 2-3 weeks          | Yes                        | Yes                    |
| Newcastle disease virus              | Yes                       | Yes/medium                    | Unknown               | 2-3 weeks          | No                         | No                     |
| Rift Valley fever virus              | Yes                       | No                            | NA                    | NA                 | No                         | Yes                    |
| Nipah and Hendra viruses             | Yes                       | No                            | NA                    | NA                 | No                         | NA                     |
| Classical swine fever virus          | Yes                       | Yes/medium                    | Unknown               | 6 weeks            | Yes                        | Yes                    |
| Venezuelan equine encephalitis virus | Yes                       | Yes/medium                    | NA                    | 3 days             | No                         | Yes                    |
| African swine fever virus            | Yes                       | No                            | NA                    | NA                 | No                         | NA                     |
| Rinderpest virus                     | Yes                       | Yes/high                      | 5 years               | 3 weeks            | Yes                        | Yes                    |
| Eastern equine encephalitis          | Yes                       | Yes/medium                    | 6 months              | 6 weeks            | No                         | No                     |

NA: Not applicable

<sup>1</sup> OIE List A or B disease and/or exotic to the United States

<sup>2</sup> Availability of commercial vaccine that has appropriate profile for control and eradication

<sup>3</sup> Duration of immunity (long duration is desirable)

<sup>4</sup> Onset of immunity (short onset is desirable)

<sup>5</sup> Availability of rapid field diagnostic test

<sup>6</sup> Stockpile needed if commercial or experimental vaccines are available

<sup>19</sup> Ibid., p. 34.

## Appendix 8. Animal Biological Threat Agents and Research Priorities<sup>20</sup>

| Agent                             | Research Need  | Priority | Rank |
|-----------------------------------|--|----------|------|
| Foot-and-mouth disease            | Define efficacy of current vaccines to be used as emergency vaccines to control FMD outbreaks  | H        | 1    |
|                                   | Increase efficacy of current vaccines with new adjuvant <i>systems</i> for use as emergency vaccine  | H        |      |
|                                   | Determine virus-host interactions critical for disease development and spread to develop novel control methods                               | M        |      |
|                                   | Identify new vaccine formulations or biotherapeutics to decrease the time to onset of immunity   | H        |      |
|                                   | Conduct research to develop vaccines designed to prolong duration of immunity to a minimum of 1 year   | M        |      |
|                                   | Identify conserved genetic and proteomic elements across the seven serotypes of FMDV that are potential target for cross-protective immunity | H        |      |
|                                   | Identify relevant T cell epitopes to modulate I cell immune responses  | M        |      |
|                                   | Identify new product delivery systems to increase the stability of the vaccine virus, leading to cost-effective FMDV vaccination programs    | M        |      |
|                                   | Develop <i>single-dose</i> vaccines that can be easily administered on a mass scale  | H        |      |
|                                   | Develop marker vaccines manufactured without the need of infectious virus seeds and allow differentiation of infected and vaccinated animals | H        |      |
|                                   | Develop effective antivirals that can prevent infection, decrease susceptibility to infection, and decrease viral shedding                   | H        |      |
|                                   | Develop companion diagnostics to marker vaccine  | H        |      |
|                                   | Identify mechanisms to induce broad protective immune responses  | H        |      |
| Highly pathogenic avian influenza | Establish efficacy profile of existing vaccines (onset and duration of immunity, effect on shed/spread)                                      | L        | 2    |
|                                   | Develop vaccines and companion serological diagnostic tests that will allow differentiation of vaccinated from field-exposed birds.          | H        |      |
|                                   | Develop AI vaccines that will allow mass immunization via feed, water or aerosol sprayers thus eliminating individual injection              | H        |      |
|                                   | Develop AI vaccines that will cross protect against the different subtypes, ensuring the availability of ready vaccines in disease outbreaks | M        |      |
|                                   | Develop ELISA-based serological tests that can be automated and provide predictability of flock immunity from AI vaccines                    | L        |      |
|                                   | Develop vaccine challenge models that will predict efficacy of new vaccines against both LP and HP AI viruses                                | M        |      |
|                                   | Improve sensitivity of RT-PCR procedures for detection of AI infections and differentiate H and N subtype in less than 8 hours               | M        |      |
|                                   | Rapid pen-side screening test for detection of AI virus in birds to allow simultaneous identification of H and N subtypes                    | M        |      |
|                                   | Develop improved models to predict which LPAI viruses can become HPAI viruses  | M        |      |
|                                   | Fund international effort to collect and sequence AI viruses with specialized bioinformatics technologies                                    | M        |      |
|                                   | Develop a global AI molecular epidemiological map based on wild bird and poultry AI viruses  | L        |      |

<sup>20</sup> Ibid., p. 35.

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| <b>Agent</b>                         | <b>Research Need</b>  | <b>Priority</b> | <b>Rank</b> |
|--------------------------------------|---|-----------------|-------------|
| Rift Valley fever                    | Determine efficacy of RVF MP-12 vaccine developed by U.S. Army to protect cattle against RFV challenge  | H               | 3           |
|                                      | Develop rapid diagnostic tools compatible with the standard systems currently in use for look-alike diseases  | H               |             |
|                                      | Develop effective methods for widespread vaccination of wildlife  | H               |             |
|                                      | Develop test model systems for rapid detection & response: GIS, remote sensing, and methods for rapidly analyzing data  | M               |             |
|                                      | Research molecular characterization of virulence factor, vector competency, host range specificity, and tissue tropism  | M               |             |
|                                      | Characterize susceptibility of EU cattle breeds and characterize protective immunity in African cattle breeds that exhibit disease resistance                 | M               |             |
|                                      | Determine vector capacity of native mosquito species in North America   | L               |             |
|                                      | Rationally design new vaccines that are marked, are safe, prevent colonization of target tissues, and significantly reduce viremia                            | H               |             |
| Exotic Newcastle disease             | Develop marker vaccines that will allow differentiation of vaccinated poultry from exposed birds  | H               | 4           |
|                                      | Develop reverse genetic system for marker vaccine development with method for gene substitutions and mutations to study Exotic Newcastle disease pathogenesis | H               |             |
|                                      | Improve mass application methods to achieve consistent immunization effects   | M               |             |
|                                      | Develop vaccine strains that can be inoculated <i>in ovo</i> as a possible approach to mass vaccine application   | M               |             |
|                                      | Develop diagnostic tests to differentiate infected from vaccinated birds  | H               |             |
| Venezuelan equine encephalitis virus | Complete the characterization of the virus; for example, virulence determinants, potential for establishment after aerosol exposure (bioterrorism)            | H               | 5           |
|                                      | Understand the natural biology of VEE; for example, environmental persistence and reintroduction mechanisms   | H               |             |
|                                      | Identify environmental factors that are correlated with the distribution of epizootic VEE virus strains   | M               |             |
|                                      | Develop equine vaccine that is cross protective against VEE epizootic variant strains   | H               |             |
|                                      | Develop pen-side virus detection test that can differentiate all major equine encephalitis viruses (for example, Eastern, Western, and West Nile)             | H               |             |
|                                      | Evaluate efficacy of biotherapeutics (for example, haptens, CpGs, and Poly IC) in enhancing cross-protective immunity of vaccines                             | M               |             |
|                                      | Develop remote sensing and geographic information systems to identify locations of potential VEE emergence  | M               |             |

## Appendix 9. Working Group Presentations

### *Dr. T. G. Ksiazek, “Rift Valley Fever Dx”*

The entire presentation is included as a PowerPoint file on the enclosed CD. Topics included the following:

- Targets for diagnosis (animal, human, vector, environmental)
- Historical diagnosis (virus isolation, serology)
- Last 20 years of RVF diagnosis (acute, surveillance)
- Short- and long-duration viremia
- Antigen and IgM capture assays
- Summary of data indicating amplifying hosts or reservoirs
- RVF diagnosis in Saudi Arabia
- Diagnostic limitations
- Molecular diagnosis
- Overall approach (serology, molecular systems)

### *Dr. C. J. Peters, “Rift Valley Fever”*

The entire presentation is included as a PowerPoint file on the enclosed CD. Topics included the following:

- Why is RVFV a concern?
- Arbovirus diseases that move around
- Possible control modalities for RVFV
- Phleboviruses
- Principles for an RVFV vaccine
- RVFV human vaccines in use (formalin inactivated and live attenuated)
- Development of MP-12 veterinary vaccine
- RVFV veterinary vaccine candidates

***Dr. K. J. Linthicum, “Use of Remote Sensing in Formulating a Predictive Model for RVF in East Africa”***

The entire presentation is included as a PowerPoint file on the enclosed CD. Discussion included the following:

RVFV disease risk mapping may provide local public health and agricultural authorities with first-level information 2-3 months before detection of disease

- Where and when to target disease surveillance/control (methoprene, ULV) teams
- Enhance vaccination, vector control, local quarantine
- Minimize cost of surveillance over large areas
- Reduce impacts of export embargoes and quarantines

Understanding links between environmental and climate factors, biology, ecology, behavior, and disease transmission might allow international public health authorities to limit globalization by

- Predicting spatial and temporal risk
- Designing better containment or exclusion strategies
- Coordinating targeted regional disease surveillance and control efforts

Predictive models could be used to improve identification (temporally and spatially) of sites for vaccine efficacy trials or evaluation of diagnostics

***Dr. J. M. Wilson, “Rift Valley Fever: Non-traditional Surveillance and Threat Assessment”***

The entire presentation is included as a PowerPoint file on the enclosed CD. Topics included the following:

- Project ARGUS
- Social disruption markers for an uncontained bioevent
- Translocation potential
- Environmental synchronization
- Transmission competency relates to temperature
- Scalable response continuum

## Acronyms and Abbreviations

| Acronym  | Definition  |
|----------|---|
| ANSER    | Analytic Services Inc.                                      |
| APHIS    | Animal and Plant Health Inspection Service                  |
| ARS      | Agricultural Research Service                               |
| BSL-3    | Biological Security Level 3                                 |
| CDC      | Centers for Disease Control and Prevention                  |
| ELISA    | enzyme-linked immunosorbent assay                           |
| IgG      | immunoglobulin G  |
| IgM      | immunoglobulin M  |
| KSU      | Kansas State University                                     |
| NABC     | National Agricultural Biosecurity Center                    |
| OIE      | Office of International Epizootics                          |
| PCR      | polymerase chain reaction                                   |
| RNA      | ribonucleic acid  |
| RT-PCR   | reverse transcriptase polymerase chain reaction             |
| RVF      | Rift Valley fever   |
| RVFV     | Rift Valley fever virus                                     |
| USAMRIID | U.S. Army Medical Research Institute of Infectious Diseases |
| USDA     | U.S. Department of Agriculture                              |
| WG       | working group   |
| WNV      | West Nile Virus   |