Strategic Research Targets to Protect American Livestockand Poultry from Biological Threat Agents

Report from the WMD Counter Measures Working Group

Animal Pathogen Research and Development Subgroup

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Foreword

This report addresses the needs for vaccines to protect animal agriculture against attacks by terrorists or others using biological agents. The report does not attempt identify all possible research needs for vaccines but to establish a set of high priority needs that when filled will protect livestock and/or humans from the most serious threats that could be introduced through infecting animals. The team that developed this report believes that it will be useful in setting priorities for research across several government agencies that have responsibility for protecting human health, our food supply, and livestock industries.

Caird E. Rexroad Jr, United States Department of Agriculture, Team Leader
Salvatore Cirone, Department of Defense
Sharon Drumm, United States Department of Agriculture
Charles Eastin, Food and Drug Administration
Peter Fernandez, United States Department of Agriculture
Cyril Gay, United States Department of Agriculture
Robert Heckert, United States Department of Agriculture
Peter Jahrling, Department of Defense
Thomas G. Ksiazek, Centers for Disease Control and Prevention
Randall Levings, United States Department of Agriculture
Don Noah, Department of Defense
Gerald Parker, Department of Defense
James Roth, Iowa State University
Scott Severin, Department of Defense
David Steele, Department of Defense
Terry Wilson, United States Department of Agriculture
Ed Nuzum, National Institutes of Health
Donna M. Gatewood, United States Department of Agriculture

Introduction

The President's National Strategy for Homeland Security calls for the development of "high efficacy vaccines" to better protect our nation from attack by the use of biological threat agents. The WMD Counter Measure Working Group was chartered by the Executive Office of the President to recommend research priorities for biodefense vaccine development. The Animal Pathogen Research and Development Subgroup was subsequently tasked to identify needs to ensure that the U.S. has an arsenal of highly efficacious vaccines to mitigate the impact of pathogen threats to animal health.

Pathogen Threats to Animal Health

Infectious diseases that pose a threat to animal and human health on an international scale are considered potential biological threat agents. The authoritative international organization for animal health is the Office of International Epizootics (OIE). The OIE has classified 15 infectious agents as "List A diseases," based on their potential for rapid spread, serious economic or public health consequence, and their impact on the international trade of animals and animal products (Appendix 1). Member countries of the OIE are required to report immediately when experiencing an outbreak with infectious agents classified as List A diseases.

In 2002, the President signed into law the Public Health Security and Bioterrorism Preparedness and Response Act, which provides for the regulation of certain biological agents and toxins by the Department of Health and Human Services (HHS) and the Department of Agriculture (USDA). The Act in subtitle B cites the Agricultural Bioterrorism Protection Act, requiring the Secretary of Agriculture to establish a list of biological (select) agents that have the potential to pose a severe threat to animal and plant health. This responsibility has been assigned to the Animal and Plant Health Inspection Service (APHIS). The Centers for Disease Control and Prevention (CDC), HHS, had previously drawn a list of 36 "select agents," 18 of which are microorganisms or toxins that pose a risk to both human and animal health. APHIS has classified these 18 microorganisms and toxins (Appendix 2) as "overlap agents or toxins." The USDA list of biological agents that pose a threat to animal health consists of 23 agents and toxins (Appendix 3). The USDA list includes 14 of the 15 OIE List A diseases (the fifteenth List A disease, Rift Valley fever, is classified as an overlap agent), five OIE List B diseases, two restricted foreign animal diseases, and two emerging paramyxoviruses.

Potential Impact of Biological Threat Agents

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 accidental outbreak of a biological threat agent. These costs are often underestimated, as demonstrated by the 1986 outbreak of mad cow disease (BSE) in the United Kingdom, which was recently reported to have cost the European Union a staggering $107 billion. For American agriculture, the impact of a biological threat agent may depend on whether the agent is foreign, emerging, or domestic. Of particular concern are those animal pathogens that have the capability of spreading disease to epizootic proportions, have complex life cycles with insect and wildlife reservoirs, or those that can be weaponized for biological warfare. In addition, the zoonotic potential of an agent should be given extra weight as the impact of an outbreak on public health and the subversion of confidence in our food supply could have a significant impact on our nation.

The Animal Pathogen Research and Development Subgroup identified ten animal pathogens from the USDA's list of select and overlap agents that could pose great harm to the nation in the event of an outbreak (see Appendix 4). The list of agents selected for this report is not exhaustive of those from which the U.S. could benefit from better vaccines. The adequacy of available vaccines, their potential role in controlling disease outbreaks, and the research needs to fill the gaps in current immunointervention strategies are presented in this document. A summary assessment of available vaccines is provided in Appendix 5. Research gaps and priorities are summarized in Appendix 6.

Vaccine Profile

Although using revision tools there is a general consensus amongst animal health officials that vaccination rather than depopulation is the most cost effective strategy in the face of a disease outbreak, there are several concerns about the safety and efficacy of vaccines on the international market today that limit their use in the control of diseases. The safety issues commonly include concerns over contamination with extraneous agents, inadequate inactivation of killed vaccines, 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, quality-controlled, and the clinical parameters used to determine efficacy. A common concern is the discrepancy in the performance of vaccines under experimental control conditions versus their actual performance in the field.

Vaccines for biological threat agents present additional challenges as they must not only prevent clinical disease, but their efficacy profile must enable control and eradication strategies without interfering with the ability of a country to obtain and maintain OIE infection-free status. Accordingly, vaccines for biological threat agents must be designed

to prevent or significantly reduce the shed and spread of infection and have to be "marked" so that infected animals can be differentiated from vaccinated animals. Defining precisely the efficacy profile of these vaccines will be critical if they are to be used effectively in an eradication campaign; for example, the minimum age that the animals can be vaccinated, the onset of immunity (OOI), and duration of immunity (DOI). Convenience and biosecurity also needs to be considered, including innovative delivery systems, the number of doses, and potential combinations with other vaccines.

An assessment of the vaccine profile for the ten biological threat agents selected for this report is provided in Appendix 5. There are no licensed commercial vaccines available for three of the ten agents discussed in this report. Although additional clinical studies are needed to fully characterize the profile of the existing vaccines, all (with the exception of Rinderpest) need improvement for optimum control and eradication. Notwithstanding, the Animal Pathogen Research and Development Subgroup recommends that the commercial and experimental vaccines available for some of the biological threat agents in this report be stockpiled for defensive purposes.

Manufacturing and Private Industry

The development of vaccines for infectious diseases that are regulated for their impact on trade represents a particular challenge for private industry since the sales potential for these products (including their companion diagnostic tests) are totally dependent on Government decisions. Thus, 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.

The development of highly efficacious vaccines that have the profile to control and eradicate animal pathogens will require an investment in cutting edge technologies. However, since new technologies are expensive industry cannot justify the investment if there is only a small market for their product. Vaccines for foreign animal diseases do not have any market in the U.S. until the disease is introduced. Vaccines for use in minor species or for diseases of minor importance frequently do not justify the investment in research and development.

The cost of production may also be prohibitive if the manufacture of the vaccine requires dedicated runs or cannot be integrated into high throughput manufacturing facilities. Contract manufacturers with state-of-the-art production facilities may need to be considered in the U.S. to ensure the containment of biological threat agents during production. Foreign manufacturers will need to be scrutinized for their ability to prevent the contamination of their product with extraneous agents. Regulatory requirements are not uniform across countries and consideration needs to be given to good manufacturing practice (GMP) requirements where the product will be manufactured and an assessment

of the cost against the level of quality assurance needed to ensure the final product is safe, pure, potent, and efficacious.

Overcoming these challenges is no guarantee that a vaccine will be used or that contracts to manufacture and build an arsenal of highly efficacious vaccines will be forthcoming. Thus, forming key alliances between the Government and the private sector through formal contractual agreements may be necessary to mitigate the risks biological threat agents pose to our nation.

Strategic Targets for Vaccine Research

New technologies hold tremendous promise for the development of safer and more effective vaccines, particularly for foreign animal diseases and for diseases for which conventional killed and modified live vaccine approaches have not been satisfactory. The effective use of these new technologies will require investing in basic research to provide a thorough understanding of the molecular basis of pathogenesis and the mechanisms of protective immunity.

The research needed for mitigating the impact of agroterrorist threats to animal agriculture is in many ways different from the research needed to mitigate the bioterrorist threats to human health. An important component to reducing the impact of bioterrorism on humans is to develop technologies to reduce disease symptoms and enhance survival. For foreign animal diseases, there is much less concern about symptoms in an animal and greater interest in eliminating the disease from the entire animal population as quickly as possible. This changes the research priorities for animal health with greater emphasis placed on epidemiology and immunointervention tools to control and eradicate infectious diseases from the U.S. animal population. Hence, strategic research targets for animal health include the development of new technologies to enable the rapid and accurate detection of infection, preventing or slowing disease transmission, and tracing animal movements and contacts to obtain disease-free certification and facilitate exports and trade.

New Vaccine Technologies

There are over 2,000 vaccines currently approved by the USDA Center for Veterinary Biologics (CVB) for use in domestic animals. These vaccines meet the requirements for purity, potency, safety, and efficacy prescribed in Title 9 in the United States Code of Federal Regulations. Nearly all of the presently licensed vaccines are conventional vaccines containing either killed or modified live whole bacteria or viruses. Most of the killed vaccines contain either aluminum hydroxide or oil and water adjuvants. Recently, vaccines have been approved with newer adjuvants or with antigens produced using biotechnology approaches. Recent advances in molecular biology, immunology,

microbiology, genetics, and understanding microbial pathogenesis have lead to the development of a wide variety of new approaches for developing safer and more effective vaccines.

The newer vaccine technologies that do not use the intact infectious agent provide the opportunity to safely manufacture vaccines for foreign animal diseases without the concern of accidental release of the infectious organism from the manufacturing facility. These new technologies include novel vaccine design such as subunit vaccines, gene deleted vaccines, live vectored vaccines, DNA mediated vaccines (sometimes coupled with subunit or killed conventional vaccines for boosting primed vaccinates), transgenic plants and plant viruses expressing immunizing antigens; novel adjuvants that target specific immune responses important for eliciting protection against specific types of pathogenesis; and slow or pulse release of antigens.

The goals for new and next generation vaccines are to have improved efficacy, improved safety, and/or increased safety or efficiency of production and therefore reduced risks or costs. Aspects of vaccine efficacy which are targeted for improvement include: induction of cell-mediated immunity, induction of protection of mucosal surfaces, ability to overcome maternal antibody interference, increased protection to heterologous challenge, ability to protect immunosuppressed, immunocompromised, or pregnant animals, rapid induction of immunity, and prolonged duration of immunity. Aspects of vaccine safety that researchers are striving to improve include: deletion of immunosuppressive or inflammatory vaccine components, alternative routes of administration that do not require intramuscular or subcutaneous injection, safety in immunocompromised animals, reduction in adverse side effects, reduction of the number of doses required and improved safety during the manufacturing process. The biotechnological approaches to developing vaccines will also allow the development of marker vaccines to differentiate vaccinated from infected animals and improving delivery systems.

Biotherapeutics to Reduce Disease Transmission

Biotherapeutic approaches to reducing disease transmission include the use of antivirals, antiparasiticides, antibacterials, and immunomodulators. Many effective antibacterials and antiparasiticides already exist. Development of new antiviral and immunomodulator drugs is an active area of basic research. These types of compounds hold promise for reducing the time it takes to induce protective immunity and reducing the transmission of infectious agents and clinical signs. There are inherent problems with using these approaches to control foreign animal disease outbreaks. The compounds typically have a short duration of action so that they must be administered frequently to maintain resistance to disease. They often have side effects and they must pass an exhaustive and expensive regulatory approval process for use in food producing animals. An advantage of the use of these types of compounds as compared to vaccines for the control of a foreign animal disease outbreak is that they are less likely to interfere with diagnostic testing and they do not compromise a country's disease free status with the OIE.

Development of safe and effective antiviral and immunomodulator compounds that would be useful to help control a foreign animal disease outbreak would likely require extensive investment in basic research over a period of many years.

Investments in Animal Pathogen Research

One challenge for developing vaccines and biotherapeutics against foreign and emerging diseases is that relatively few scientists in the U.S. are working in this area of research. There are few opportunities for veterinarians to pursue careers in research and postdoctoral positions in animal pathogen research in Government laboratories and universities are lacking. Investing in our infrastructure will also be critical as there is limited veterinary medical high containment laboratory and animal facilities in the U.S. With limited resources, strategic collaborations between Government laboratories, universities, and the private sector is a necessity in animal pathogen research. The estimated FY 2004 budget and FY 2005 requested increases by the pertinent Government agencies conducting research on infectious diseases are provided in Appendix 6.

Recommended Control Strategies and Research Needs

The following provides an assessment of the research needed to increase our arsenal of intervention tools to effectively control and eradicate outbreaks of the biological threat agents selected for this report. Appendix 7 ranks each agent according to their potential threat on the nation, summarizes the research needs by agent, and prioritizes the research into low, medium, and high categories.

Foot-and-Mouth Disease Virus

Description

Foot-and-Mouth Disease virus (FMDV) belongs to the family Picornaviridae. The Picornaviridae are sometimes referred to as the disease virus group. Picornaviruses are among the most diverse (more than 200 serotypes) and oldest-known viruses. FMDV was one of the first viruses to be recognized.

The virions are not enveloped and contain a single strand of positive-sense RNA of coding for the 4 structural proteins (genes 1A-1D), and the eight non-structural proteins (genes L, 2A-C and 3A-D). FMDV are classified according to their genome organization. There are 7 serotypes (Types A; C; O; SAT-1, SAT-2, SAT-3; Asia-1).

The antigenicity of FMDV involves continuous and discontinuous neutralizing epitopes on one or more of the exposed parts of the capsid proteins, particularly VP1. There is no cross immunity between serotypes. There are more than 60 subtypes; some of them are strongly divergent.

Disease Transmission

FMDV is one of the most contagious animal diseases. Cattle are the most susceptible of the domesticated species to FMDV; as little as 10 tissue culture infectious doses are required to establish infection by inhalation. Cattle are therefore the principal indicators of the disease. Pigs are important amplifiers because their capacity to excrete large quantities of virus. Sheep are maintenance hosts since they display very mild symptoms.

FMDV infection of susceptible animals in the field occurs primarily through the upper respiratory tract by inhalation of airborne virus from an infected animal. Aerosol transmission usually occurs with animals in close proximity. However, there is circumstantial evidence that animals may be infected from several yards to many miles downwind from a source of infection. Reports of field outbreaks indicate that convalescent cattle may transmit the disease when introduced into an FMD-free herd. The role of carrier animals in the transmission has never been demonstrated experimentally in cattle and sheep. Indirect transmission is important because the virus can retain infectivity for a considerable time in the environment. The animals may get their initial throat infection of FMDV by ingesting contaminated forage, grain, animal products or water, or by licking contaminated objects. The virus is inactivated in the meat of carcasses that undergo the normal post-slaughter acidification processes, but it persists for a very long time in frozen or chilled lymph nodes, bone marrow and residual blood clots. It also retains infectivity in uncooked, salted and cured meats, and unpasteurized dairy products.

Vaccination

The endorsement by the European Union in the last decade of a non-vaccination policy to control FMDV outbreaks led to a decrease or elimination of vaccination in many countries. The rationale for this policy was based on epidemiological assessments that suggested that vaccination would not have stopped the spread of recent epidemics any faster than “stamping out” or depopulation strategies; and the prohibitive cost associated with the additional waiting period imposed on countries that vaccinate before they can attain OIE infection-free status and are allowed to export animals and their products. Although non-vaccination policies are meant to protect food animal production on a global scale, the cost to individual countries can be significant. In the case of developing countries, where food animals represent a major segment of the economy, depopulation is not a realistic option. In the industrial world, it has become increasingly clear that the economic impact of non-vaccination policies is not equitable and affects individual

countries disproportionately and is far more costly than previously thought. For example, the cost of the FMDV outbreak in the United Kingdom (UK) in 2001 was estimated to be 9.2 billion USD. Industrial nations are also recognizing the hidden costs of non-vaccination policies with the slaughter of an estimated 6.24 million animals in the UK in 2001, resulting in the loss of bio-diversity and native livestock populations. The cost of eradicating FMDV from the U.S. in the event of an outbreak using a non-vaccination strategy can easily be estimated to be a 100-fold higher than the UK, considering that the U.S. has a 100 million head of cattle, a rich population of wild-life, and exotic animals in parks and zoos throughout the country.

The OIE has recently endorsed vaccination as a control measure during an outbreak and has shortened the time required to obtain disease free status if vaccination is used in a control strategy. This official endorsement stemmed both from the 'lessons learned' from the UK outbreak and the recent introduction of serologic tests that differentiate vaccinates from infected animals.

FMDV vaccines are the leading veterinary biological product sold worldwide with 284 million USD in sales in 2000. There are several FMD vaccines available, reflecting the need to provide protection against all serotypes. FMD vaccines are presently available from major animal health pharmaceutical companies. All vaccines are derived from viruses grown in tissue culture, chemically inactivated, and adjuvanted. The adjuvant for ruminants is commonly aluminum hydroxide with or without saponin, whereas pig vaccines require oil adjuvants. The vaccines are usually marketed as monovalent vaccines (e.g., type A or type O) but it is important to know the subtype or strain of virus used in the vaccine (e.g., Type O, PanAsia strain). This allows decisions to be made to match the most appropriate vaccine to the strain responsible for an outbreak. In countries where more than one serotype circulates, multivalent vaccines are used. There is some variation in the duration of immunity provided by the different commercial vaccines. The vaccine regimen for FMD vaccines requires two-doses one-month apart, followed by booster doses every 6 months. New diagnostic tests have recently been developed based on the absence of specific non-structural proteins in the inactivated vaccines that should allow the differentiation of infected and vaccinated animals.

Limitations of Current FMDV Vaccines

Limitations of the current vaccine(s) include the injection route of administration, a multiple dose regimen, and the inability throughout much of the world to differentiate between vaccinated animals and those infected with the disease. Currently, vaccination of healthy animals is possible but immunity is virus type-specific, there is no cross-protection, and it is short-lived. Current vaccines only provide six month's protection at best. There is also the danger that vaccinated animals, although protected against developing the disease, may become carriers if exposed to new infections of the virus. Critical factors for future FMD vaccines are broad serotype coverage, prolonged

immunity with a single injection, a marker vaccine, and rapid availability of many doses in the face of an outbreak.

Vaccines in a Control Strategy

Because FMD is one of the most infectious diseases known to man and the U.S. has millions of susceptible animals, an extensive emergency vaccination program in our FMD control and eradication strategy must be considered. In that scenario, emergency vaccination (ring vaccination) of all susceptible contact farm animals (cattle, pigs, sheep, goats) should be implemented immediately upon recognition of an outbreak. Sufficient doses of vaccine should be available to rapidly create a "buffer" region around the infected zones. At least 80 percent of the susceptible animals must be vaccinated. Using current vacccines, two doses of vaccine must be administered within the first month, followed by additional doses at 6 months and 1 year. The U.S. could allow vaccination of special breeds and FMD susceptible animals in wildlife reserves and zoos, provided that strict movement controls on these animals can be maintained. Because the current vaccines provide no cross-protection against the different serotypes (and potentially some of the divergent genotypes), the U.S. should continually evaluate the types and amounts of available vaccines in the North American Vaccine Bank.

Research Needs

- Define efficacy of current vaccines to be used as emergency vaccines to control FMD outbreaks.
- Increase efficacy of current vaccines with new adjuvant systems for use as emergency vaccine.
- Determine the virus-host interactions that are critical for disease development and spread (including the host's receptor molecules that the virus uses to initiate its attack on susceptible cells), and attempt to use this information to develop novel control methods.
- Identify new vaccine formulations or biotherapeutics to decrease the time to onset of immunity.
- Conduct research to develop vaccines designed to prolong duration of immunity to a minimum of 1 year.
- Identify conserved genetic and proteomic elements across the 7-serotypes of FMDV that are potential target for cross-protective immunity.

- Identify relevant T cell epitopes to modulate T cell immune responses.
- Identify new product delivery systems to increase the stability of the vaccine virus leading to cost effective FMDV vaccination programs.
- Develop single-dose vaccines that can be easily administered on a mass scale.
- Develop marker vaccines that can be manufactured without the need of infectious virus seeds and allow differential diagnosis of infected and vaccinated animals.
- Develop effective antivirals that can prevent infection, decrease susceptibility to infection, and decrease viral shedding.
- Develop companion diagnostics to marker vaccine.
- Identify mechanisms to induce broad protective immune responses.

Avian Influenza Virus (Highly Pathogenic)

Description

Influenzavirus A virus of the family Orthomyxoviridae causes avian influenza (AI). The virion is enveloped and contains 7 segments of linear negative-sense single stranded RNA. AI viruses are subtyped into 15 hemagglutinin subtypes (H1-15) and 9 neuraminidase subtypes (N1-9). Low pathogenicity (LP) AI viruses can be any combination of the 15H and 9N subtypes, while highly pathogenicity (HP) AI viruses are only H5 or H7 subtypes but can be any of the 9 N subtypes (i.e. H7N7, H7N1, H5N2, H5N1, etc.).

HPAI impacts international trade by inhibiting exports from an infected country. With some countries, H5 and H7 LPAI will also inhibit trade. Some H5 and H7 LPAI strains have shown the ability to mutate and become HPAI viruses.

AI is a potential zoonotic agent and on rare occasion causes disease in humans. A 57-year-old veterinarian who recently visited a poultry farm in the Netherlands affected by the (H7N7) strain died of acute respiratory distress syndrome. AI does not spread efficiently from humans to humans.

Disease Transmission

Feces from infected birds are the most important source of AI virus. Shedding occurs 7-14 days post infection and may continue for up to 4-weeks.

Wild birds, especially ducks and shorebirds, are reservoirs of LPAI viruses. The latter can have up to a 60% infection rate. AI viruses from wild birds can spill over into domestic poultry, especially those raised outdoors. Among domestic poultry species, turkeys are more commonly infected than are chickens. In recent years, H7N2 LPAI has been a problem in the live poultry markets of the Northeastern U.S. On several occasions, AI has been transmitted to commercial poultry from these live poultry markets.

Sporadic outbreaks of LPAI occur in the U.S. in commercial poultry flocks on an annual or semi-annual basis. These are dealt with on a State-by-State basis usually through biosecurity enhancements, controlled marketing of the flocks and in the case of turkeys, some use of inactivated vaccines. Our last HPAI outbreak was in Pennsylvania-Maryland-Virginia during 1983 - 84. Depopulation was used to eradicate the disease.

Vaccination

Vaccination against AI is not routine in domestic poultry since AI is not endemic in the U.S. There is no economic advantage to national AI vaccination because complete protection from AI using current technology would require 15 different vaccines, one for each hemagglutinin subtype.

Current AI vaccines prevent disease and death. They also increase resistance to infection and reduce replication and shedding of the field viruses. They do not completely eliminate asymptomatic virus replication in the respiratory and/or gastrointestinal tracts. Because of potential re-assortment of AI viral genes, live whole AI virus vaccines are not used.

Currently, USDA has conditional licenses for inactivated AI vaccines for most of the 15-hemagglutinin subtypes. Full-licensure has been granted for a fowl pox recombinant containing the H5 gene from AI and an inactivated H5N2 whole AI virus vaccine. Conditional licensure is available for an inactivated H7N2 whole AI vaccine. Use of H5 and H7 AI vaccine is controlled by USDA, requiring an emergency need and USDA approval before field application.

In the U.S., the most frequent AI vaccination has been in turkey breeders raised in areas with significant swine populations infected with H1N1 or H1N2 influenza. In 2001, 2.8 million doses of inactivated H1 vaccine were used in turkey breeders in the U.S. to prevent devastating drops in egg production, while only 677,000 doses of H6N2 inactivated vaccine were used in egg laying chickens in California.

Limitations of Current AI Vaccines

- Current vaccines do not cross-protect against the different hemagglutinin subtypes.
- Vaccines must be produced on demand and in large quantities with the correct hemagglutinin subtypes.
- Vaccination complicates trade in poultry and poultry products with some countries refusing imports of such products from countries with AI vaccination.
- Differentiation of vaccinated from field exposed poultry is not currently possible using inactivated AI vaccines since all vaccinated and field exposed birds are positive for anti-AI antibodies on the agar gel precipitation (AGP) or specific hemagglutinin inhibition (HI) tests.
- Fowlpox-AI-H5 recombinant vaccine is ineffective in poultry previously exposed to or vaccinated with fowl pox vaccines. This is common in most egg laying chickens and chicken and turkey breeders.
- All current AI vaccines require injection of individual birds, which is expensive.

Vaccines in a Control Strategy

Because HPAI is a highly infectious disease with zoonotic potential, an emergency vaccination program for the control and eradication of HPAI should be considered. A critical factor is mass vaccine administration as the delivery to individual birds is expensive and inefficient. To be most effective, a strategy based on "differentiating infected from vaccinated animals" (DIVA) should be implemented by formulating vaccines with the correct hemagglutinin (H5 or H7) but a different neuraminidase subtype than the AI implicated in the outbreak.

Research Needs

- Establish efficacy profile of current vaccines (OOI, DOI, effect on shed/spread).
- Develop vaccines and companion serological diagnostic tests that will allow differentiation of vaccinated from field-exposed birds.

- Develop AI vaccines that will allow mass immunization via feed, water or aerosol sprayers thus eliminating individual injection.

- Develop AI vaccines that will cross protect against the different subtypes, ensuring the availability of ready vaccines in disease outbreaks.

- Develop ELISA-based serological tests that can be automated and provide predictability of flock immunity from AI vaccines.

- Develop vaccine challenge models that will predict efficacy of new vaccines against both LP and HP AI viruses.

- Improve sensitivity of real time RT-PCR procedures for detection of AI infections in poultry flocks and differentiate hemagglutinin and neuraminidase subtype in less than 8 hours.

- Develop a rapid "pen-side" screening test for detection of AI virus in birds based on conserved AI internal proteins and allow simultaneous identification of H and N subtypes.

- Develop improved models to predict which LPAI viruses can become HPAI viruses.

- Fund international effort to collect and sequence AI viruses with specialized bioinformatics technologies.

- Develop a global AI molecular epidemiological map based on wild bird and poultry AI viruses.

Exotic Newcastle Disease (END) Virus

Description

Exotic Newcastle disease (END) is a contagious and fatal viral disease affecting all species of birds. Previously known as velogenic viscerotropic Newcastle disease (VVND), END is one of the most infectious diseases of poultry worldwide. END is so virulent that many birds die without showing any clinical signs. A death rate of almost 100 percent can occur in unvaccinated poultry flocks.

END virus is a member of the genus Rubulavirus in the family Paramyxoviridae. The virions are enveloped and contain a single linear strand of mostly negative-sense RNA.

Only minor antigenic variations, differentiated with monoclonal antibodies, have been found among Newcastle disease virus (NDV) isolates recovered from the first reports of ND in 1926 when compared to the most recent isolates like those from the 2002-2003 outbreak in the U.S. However, the majority of antigens are shared among all NDV strains, which are recognized as a single serotype referred to as avian paramyxovirus type-1 (APMV-1). All APMV-1 isolates will cross-protect against challenge with any other APMV-1 isolate.

APMV-1 is classified into three major pathotypes: lentogenic, mesogenic and velogenic. Lentogenic viruses produce a mild respiratory disease or subclinical infections, mesogenic viruses produce more severe respiratory disease with mortality only in very young birds, and velogenic viruses produce severe disease with high mortality and neurologic or systemic lesions. Lentogenic viruses can cause significant disease in chickens and turkeys when associated with secondary bacterial pathogens. Lentogenic APMV-1 cause endemic disease in poultry of the U.S., but mesogenic and velogenic APMV-1 are considered exotic. The current (2003) California outbreak is the result of velogenic NDV.

Disease Transmission

END virus can infect a wide variety of bird species. Some species do not show any signs of disease when infected. Parrots and other psittacine birds are of special concern because they can carry END virus and show no clinical signs. Many other birds can become infected with END but have not been associated with the transmission of disease to chickens and turkeys. The most likely source of END is infected chickens and turkeys. Most infected chickens and turkeys eventually die from this disease but there is a period before they succumb when they can easily spread the virus.

The END virus is hardy and can easily survive on the feet, hands, and clothes of humans. In addition, it can survive in the eyes and in nasal passages of people who have been in contact with infected birds.

The END virus is excreted in feces and from the respiratory tract as an aerosol. The virus can easily contaminate feed, water, footwear, clothing, tools, equipment, and the environment. Fertile eggs laid by infected hens can carry virus although they rarely hatch. However, the distribution of hatching eggs from an infected flock can carry the virus to susceptible birds.

Vaccination

Newcastle disease vaccination is widely practiced in the U.S. with the majority of commercial broiler breeders, layers and turkeys receiving multiple vaccinations during

their lifetime. The live virus NDV vaccines are abundant and inexpensive. Inactivated virus vaccines are used on a more limited basis for layers, breeders, and some turkeys. Initial vaccination is with a live low virulence lentogenic vaccine followed by either repeated live lentogenic or inactivated vaccine. Broilers receive only the live virus vaccines. Inactivated vaccines licensed for use in pigeons are used widely, particularly in racing pigeons. Vaccines licensed for use in chickens and turkeys are used in emergency with other Galliformes but should not be used in other bird species.

Currently licensed vaccines provide the appropriate antigenic spectrum. Successful field vaccination must also overcome any existing maternal immunity, concurrent infections (some being immunosuppressive), an adequate antigen dose, and mass vaccine delivery failures (individual application is used only with inactivated vaccine). More aggressive vaccination programs with live virus result in "vaccine reactions" or respiratory disease with economic losses in addition to increased vaccine cost.

Currently licensed ND vaccines will induce protection to disease from an exotic END virus challenge.

Limitations of Current ND Vaccines

Vaccination is practiced to prevent or minimize economic losses from endemic strains of the APMV-1 (lentogenic NDV). Current vaccination programs are not optimized for protection for the more virulent (i.e., exotic) NDV strains. In countries where END is endemic, live vaccines are used at higher dosage and more frequently than their routine use in the U.S. Live vaccines are often applied in combination with inactivated vaccines. These intensive programs to control END increase the cost of vaccination and the more intense vaccination regime causes productivity losses from increased vaccinal reactions.

Vaccines in a Control Strategy

Because END is one of the most infectious diseases of poultry, is extremely virulent (100 percent mortality in non-vaccinated flocks), and lentogenic NDV viruses are endemic in the U.S., extensive emergency vaccination of commercial flocks should be considered to prevent clinical disease and limit the spread of infection. Since commercial vaccines have not been optimized to provide protection against END, vaccination programs should be developed to maximize the performance of existing vaccines.

Research Needs

- Develop marker vaccines that will allow differentiation of vaccinated poultry from exposed birds.

- Establish a reverse genetics system for development of live marker vaccines that provide a method for gene substitutions and creation of mutations to contribute to ongoing END pathogenesis studies.

- Improve mass application methods to achieve consistent immunization effects.

- Develop vaccine strains that can be inoculated in ovo is a possible approach to mass vaccine application.

- Develop diagnostic tests to differentiate infected from vaccinated birds.

Rift Valley Fever Virus

Description

Rift Valley fever (RVF) is an acute vector-borne zoonotic disease of domestic ruminants in Africa. A member of the genus Phlebovirus in the family Bunyaviridae causes the disease. The virions are enveloped and contain circular single stranded RNA. The disease occurs in climatic conditions favoring the breeding of mosquito vectors. The disease is most severe in sheep, goats and cattle. Microscopically, hepatic necrosis is the most obvious lesion of RVF in both animals and humans. The acute form is most common in young animals where it causes high mortality. Abortion is often the only sign seen in adults. Although older non-pregnant animals are susceptible to infection, they are more resistant to clinical disease. There is considerable variation in the susceptibility of animals to different RVF genotypes. Animal breeds that are exotic to Africa or are from areas where RVF is not endemic tend to be more susceptible.

Humans are susceptible to infection by handling infected material and through transmission by mosquito vectors. Infection of humans by vectors is a striking feature in countries with a relatively small population of animal hosts. In such areas, RVF may be recognized first in humans. RVF has caused serious disease in laboratory workers and must be handled with high level biosecurity.

RVF was first described in 1930 in the Rift Valley of Kenya. The disease has since occurred irregularly in Kenya every 3- to 10-years. Egypt experienced a severe epizootic in 1997 that resulted in huge losses among the domestic animal populations and caused significant human disease. The total morbidity was thought to be in the hundreds of thousands, and the resources of the hospitals in the affected areas were severely strained by the numbers of cases presenting daily. Most cases were thought to arise from

mosquito bites, but many of the human cases followed close contact with infected animals, and the aerosol route of infection appeared to be responsible. In September 2000, a RVF outbreak was reported in Saudi Arabia, representing the first Rift Valley fever cases identified outside Africa.

Disease Transmission

RVF generally occurs during years of unusually heavy rainfall and when localized flooding occur. The excessive rainfall facilitates mosquito eggs 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 new generations of infected mosquitoes may hatch from their eggs. This provides a durable mechanism for maintaining the virus in nature, as the eggs of these mosquitoes may survive for periods of up to several years in dry conditions.

Once livestock is infected, a wide variety of mosquito species may act as the vector for transmission of RVF virus and can spread the disease. A different species of mosquito may prove to be the predominant vector in separate regions. In addition, it is possible that other biting insects can transmit RVF virus.

Vaccination of livestock in infected areas is frequently not recommended because needle reuse can transfer the virus from infected animals to naïve animals.

Vaccination

Both modified live virus and killed vaccines have previously been developed but are inadequate for veterinary or public health use. The first vaccine was attenuated by serial intracerebral inoculation of mice (Smithburn strain) and one dose was effective in providing onset of immunity within 6-7 days and duration of immunity of at least three years. However, when administered to pregnant ewes, the vaccine caused abortion and was pathogenic for man. To overcome the safety problem a cell culture inactivated vaccine was developed but this vaccine requires multiple doses to produce protective immunity and the large amounts of antigen required impacts the cost of production and the number of doses that can be produced.

The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) recently developed a mutagen-attenuated vero-cell-propagated vaccine for use in people, described as the 12th MRC-5 mutagenesis passage of ZH-548 strain RVFV (RVF MP-12). This vaccine has been tested in sheep and cattle. The vaccine causes no adverse effect in neonatal lambs, calves, or pregnant sheep or cattle. This vaccine also has the advantage that one inoculation induces rapid immunity, and as few as 10 plaque-forming

units of the virus vaccine induces protection, resulting in high yield vaccine production output. This vaccine is not commercially available.

Limitations of Current RVF Vaccines

Only the modified live Smithburn strain and inactivated vaccines are commercially available. The modified live Smithburn strain vaccine causes abortions preventing extensive vaccination of the target host population. The killed vaccines require multiple doses and efficacy is not optimum. The only source of vaccines is in Egypt and South Africa. Vaccine supply is an issue.

Vaccines in a Control Strategy

Since RVF is a vector-born zoonotic disease with severe public health consequences and domestic livestock can serve as a reservoir and amplify the virus, emergency vaccination of all susceptible animals in "vector-favored" high-risk zones should be implemented immediately in the case of an outbreak. The MP-12 experimental vaccine is the only available vaccine with the correct profile for control and eradication; thus, serious consideration should be given to implementing a full development plan to establish purity, safety, potency, and efficacy, including contracting production and stockpiling sufficient doses to enable an extensive vaccination programs. The execution of a full development plan for a modified-live veterinary vaccine is estimated to be 18-months. Until such plan can be executed, stockpiling inactivated vaccine that is commercially available in Africa may be a consideration. The number of doses to be stockpiled will require an assessment of vaccine efficacy and the expected epidemiological spread if an outbreak were to occur in the U.S.

Research Needs

- Determine efficacy of RVF MP-12 vaccine developed by U.S. Army to protect cattle against RFV challenge.
- Develop rapid diagnostic tools compatible with the standard systems currently in use for look-alike diseases.
- Develop effective methods for widespread vaccination of wildlife.
- Develop test model systems that will allow the rapid detection and response to vector-borne zoonotic diseases such as Rift Valley fever. Geographic Information

Systems (GIS), remote sensing of environmental factors, and methods for rapidly analyzing data will be important components.

- Research molecular characterization of virulence factor, vector competency, host range specificity, and tissue tropism.

- Characterize immune response in susceptible European cattle breeds and characterize protective immunity in African cattle breeds that exhibit disease resistance to RVF.

- Rationally design new vaccines that are marked, safe in all stages of cattle production, prevent colonization of target tissues, and significantly reduce viremia.

Nipah and Hendra Viruses

Description

The Nipah virus was first described during a 1998-1999 epidemic in Malaysia, which resulted in the deaths of 105 persons and the slaughter of approximately 1.1 million pigs. The Nipah virus causes serious and sometimes total encephalitis in humans and respiratory and central nervous system disease in swine and other animals. This virus and the related Hendra virus are paramyxoviruses that are distinct from any of the other established genera of paramyxoviruses and have been proposed to represent members of a new genus (Henipavirus). The Nipah virus is considered by the CDC and NIAID as a category C priority pathogen for biodefense research. Hendra virus, formerly known as equine morbillivirus, was first isolated during an outbreak of a severe respiratory illness that killed 14 horses and 1 human in Hendra, Queensland, Australia in 1994. Antibodies to Hendra virus have been detected in four species of fruit bats (Pteropus sp.) indigenous to Australia. Thus the Pteropus species are currently thought to be the natural host of Hendra virus.

Disease Transmission

Although mortality appears to be low in pigs, the virus can spread readily from pigs to humans and other animals, with serious consequences. Although the initial outbreak in Malaysia seems to be at an end, a reservoir of virus appears to exist in asymptomatic fruit bats. Antibodies to the Nipah virus cross-react with the Hendra virus.

Nipah virus infections have been seen in pigs, humans, dogs, and goats. Antibodies to the virus have been reported in dogs, cats, horses, and goats, and virus antigens were found in one case of meningitis in a horse. Sheep may also be affected. The fruit bat, commonly known as the flying fox (Pteropus spp.), is the probable reservoir host and is found in the Indian subcontinent, Southeast Asia, Australia, and some of the Pacific Islands. Nipah or Nipah-like viruses are probably widespread in Southeast Asia.

Most infections in humans are thought to occur after close direct contact with the excretions or secretions from an infected pig. Infections spread readily from infected pigs to other species. Nipah virus does not appear to be spread from human to human.

The Nipah virus outbreak in swine in Malaysia was eliminated through an aggressive slaughter and eradication program. More than one million pigs were destroyed to control the outbreak. This represented about half of the pigs in Malaysia. There is great concern that Nipah virus infection may reappear and perhaps spread to other countries in the region with less well developed infrastructure for detecting and eradicating the disease from swine. There are no vaccines for these diseases.

Vaccination

No vaccines are available for these infectious diseases

Research Needs

- Develop a marker vaccine and a companion diagnostic test that which limit spread of the infection while allowing the detection of infected animals.
- A human vaccine is urgently needed to protect humans who may have contact with pigs during an outbreak and for laboratory workers who may be exposed.
- Better define the native vectors of these viruses.
- Determine the probability of spread in intensively reared livestock (i.e., mode of transmission).

Classical Swine Fever

Description

Classical Swine Fever (CSF) is a highly contagious, economically significant viral disease of domestic pigs. The disease, which is often fatal, is characterized by fever, hemorrhages, ataxia, and immunosupression; however, the course of infection varies depending on host characteristics and the particular virus strain. CSF occurs in several forms, ranging from highly lethal to subclinical. Sub-acute and chronic forms of the disease are associated with CSFV strains of moderate and low virulence, respectively. The causative agent, CSFV, is a member of the genus Pestivirus of the family Flaviviridae along with two other viruses of significant veterinary importance, bovine viral diarrhea virus (BVDV) and border disease virus (BDV). The virion is enveloped and contains a single strand of positive sense RNA.

CSF is enzootic in Eastern Europe, Southeast Asia, South and Central America, southern Mexico, and the Caribbean. The U.S. was declared free of CSF in 1978. Recent outbreaks in countries free of the disease, including the Netherlands, Germany, England and Spain, have resulted in highly significant economic losses for their respective swine industries. Costs related to the recent outbreak in the Netherlands (1997-1998) exceeded two billion USD. A disease control policy involving slaughter of exposed animals is practiced in countries of the European Union. Controlling and eliminating the disease by this approach has proven effective, but at costs now becoming unacceptable. In addition to staggering economic losses, issues related to public acceptance, animal welfare and environmental protection are now being raised.

Disease Transmission

The most common method of transmission is direct contact between healthy pigs and those infected with CSF. The disease can also be transmitted through contact with body secretions and excrement from infected animals. Healthy pigs coming into contact with contaminated vehicles, pens, feed, or clothing may contract the disease. Birds, flies, and humans can physically carry the virus from infected to healthy pigs. Swine owners can inadvertently cause infection through feeding their herds untreated food wastes containing infected pork scraps.

Vaccination

CSF disease control programs in enzootic areas are based on vaccination of domestic pigs. Most currently used vaccines are live attenuated virus vaccines (LAV). In general, these LAV vaccines provide lifelong immunity against disease. However, and most significantly, it is not currently possible to distinguish between animals vaccinated with

LAV and animals infected with wild-type virus, making their use in conjunction with a control/eradication program problematic.

Candidate CSFV subunit marker vaccines has been developed using recombinant E2 envelope protein. E2, the major structural protein, induces neutralizing antibodies and protective immunity in infected and vaccinated pigs. Baculovirus expressed E2 glycoprotein has been used in a commercially available subunit vaccine. Unfortunately, E2 subunit vaccines are not as efficacious as traditional LAV, particularly when animals are challenged shortly after vaccination. Failure to induce rapid and efficient protective immunity precludes use of subunit vaccines during emergency disease control measures. Delivery of E2 by DNA vaccine has also been shown to induce protection in pigs, but again failed to do so in a rapid manner.

Several live vector vaccines have been developed, using vaccinia, Pseudorabies, and adenoviruses. Limited evaluation of their potency, possible preexisting anti-vector immunity and questions surrounding introduction of recombinant vaccinia or adenovirus into the swine population have discouraged their development and use.

Recently, encouraging results have been obtained resulting in a non-replicating live attenuated vaccine. These approaches need further research.

Vaccines in a Control Strategy

Vaccination should be an important component of a control and eradication strategy should an outbreak occur in the U.S. A CSF emergency use vaccine should: 1) induce a rapid protective immune response and/or reduce viral shedding and disease transmission; 2) be antigenically marked, eliciting an immune response distinguishable from that of field virus (DIVA); and 3) be easily and rapidly administered to large numbers of animals.

Research Needs

- Develop recombinant, virulence and host range-restricted, CSF viruses for use as live attenuated, antigenically marked vaccines. Currently, a LAV is the most promising option to meet these needs. LAV vaccines need to be improved to address their deficiencies described above.

- Develop a fundamental understanding of the genetic elements critical for viral pathogenesis and virulence including induction of disease, generalization of infection, tissue tropism, host range, and induction of immune responses.

- Develop methods to improve the efficacy of CSF subunit vaccines to levels obtainable with LAV vaccines. Manipulation of host responses with immunomodulators and/or use of other vectors and or antigen delivery routes are potential approaches. However, it is unlikely in the short term these will prove effective

- Develop new strategies and/or vector delivery systems for rapidly vaccinating large numbers of animals.

Venezuelan Equine Encephalitis (VEE) Virus

Description

Venezuelan equine encephalitis (VEE) is an acute vector-borne zoonotic disease that is endemic in northern South America, Trinidad, Central America, Mexico, and Florida. VEE virus is a member of the family Togaviridae, genus Alphavirus. It is closely related to Western and Eastern equine encephalitis viruses. Virions are enveloped and contain a single linear strand of positive-sense RNA. Nine variants within six subtypes (I-VI) of VEE have been identified serologically. The majority of these subtypes are enzootic strains that do not cause disease. The majority of epizootic strains that are pathogens are designated subtype I, variants A/B. These agents cause severe disease in horses, mules, burros and donkeys (Equidae) and humans. Natural infections are acquired by the bites of a wide variety of mosquitoes.

VEE outbreaks in the U.S. are rare. The highly pathogenic form of VEE has not occurred in the U.S. since 1971. Fewer than 100 laboratory-confirmed cases in humans are documented. Data from international outbreaks suggest that many infections are subclinical or mild. Unless a large-scale epidemic in horses occurs in the U.S., VEE outbreaks will have been acquired abroad or will be due to an intentional release of the pathogen.

Alphaviruses possess characteristics that make them well suited for weaponization; they can be produced in large quantities, delivered effectively via the aerosol route, and are relatively stable in the environment. VEE was previously weaponized in the 1940s by countries with biological weapons programs, including the U.S. Like many other viruses, VEE is potentially susceptible to genetic manipulation. This characteristic has proven useful in the laboratory in the development of more effective vaccines; however, it could also be exploited to produce more effective biological weapons.

Disease Transmission

Mosquitoes serve as a vector for transmission of VEE virus. VEE has a zoonotic reservoir in bats, birds, rodents, equines (horses, donkeys, mules), and certain tropical jungle mammals. Rodents and other small animals are the most important amplifiers in endemic preservation of the virus in tropical forests, swamps, and marshlands. Horses are the most important amplifier hosts in large epidemic outbreaks. The speed with which the disease spreads depends on the subtype of the VEE virus and the density of mosquito populations.

Enzootic subtypes of VEE are diseases endemic to certain areas. Generally these serotypes do not spread to other localities. Enzootic subtypes are associated with the rodent-mosquito transmission cycle. These forms of the virus can cause human illness but generally do not affect equine health.

Epizootic subtypes can spread rapidly through large populations. These forms of the virus are highly pathogenic to horses and can also affect human health. Equines, rather than rodents, are the primary animal species that carry and spread the disease. Infected equines develop an enormous quantity of virus in their circulatory system. When a blood-feeding insect feeds on such animals, it picks up this virus and transmits it to other animals or humans. Although other animals, such as cattle, swine, and dogs, can become infected, they generally do not show signs of the disease or contribute to its spread.

Vaccination

Horses in the U.S. are not vaccinated. Vaccines are available for humans and horses but most have significant limitations. Two vaccines are licensed in the U.S. for horses: 1) VEE C-84 (inactivated) and 2) VEE TC-83 (modified live virus).

Limitations of Current VEE Vaccines

Existing vaccines for VEE are derived from variants IA/B and have been shown effective in preventing disease from homologous VEE IA/B infections. However, the current vaccines do not adequately protect against the VEE IE variant, as previously demonstrated in laboratory workers vaccinated with the VEE IA/B-derived vaccine. Also, recent unprecedented outbreaks of VEE IE in Mexican horse populations indicate a need for a VEE IE vaccine. The currently used vaccines are formalin-inactivated preparations that are poorly immunogenic, require multiple inoculations, frequent boosters, and are inadequately protective against aerosol exposure to the virus. The modified live vaccine provides better protection against the aerosol challenge and has a fast onset of immunity than the inactivated vaccine but there is concern that it could revert to virulence and/or be transmitted by mosquito vectors.

Vaccines in a Control Strategy

Because equines are the primary animal species that carry and spread the highly pathogenic strains of VEE, the most effective way to prevent further spread of disease is to implement a large-scale equine vaccination program and quarantine infected equines. Controlling mosquito populations through pesticide treatments and eliminating insect-breeding sites will also enhance disease control.

Research Needs

- Complete the characterization of the virus; e.g., virulence determinants, dose and route associations, vectorial capacity, potential for establishment after aerosol exposure (bioterrorism) of humans and horses.
- Understand the natural biology of VEE; e.g., environmental persistence and reintroduction mechanisms.
- Identify environmental factors that are correlated with the distribution of epizootic VEE virus strains.
- Develop equine vaccine that is cross protective against VEE epizootic variant strains.
- Develop pen-side virus detection test that can differentiate all major equine encephalitis viruses (e.g., Eastern, Western, and West Nile).
- Evaluate efficacy of biotherapeutics (e.g., haptens, CpGs, and Poly IC) in enhancing cross protective immunity of vaccines.
- Develop remote sensing and geographic information systems (GIS) to identify locations of potential VEE emergence.

African Swine Fever Virus

Description

African swine fever (ASF) is a highly lethal hemorrhagic disease of domestic swine with mortality rates approaching 100 percent. ASF occurs in several disease forms, ranging from highly lethal to subclinical infections depending on contributing viral and host factors. Hemostatic and hemodynamic changes (hemorrhage, edema, ascites, shock) resulting from intravascular activation of coagulation are observed in dying pigs following infection with virulent strains of virus. ASFV infects cells of the mononuclear-phagocytic system, including fixed tissue macrophages and specific lineages of reticular cells; affected tissues show extensive necrosis following infection. The abilities of ASFV to replicate and efficiently induce marked cytopathology in these cell types in vivo appear to be critical factors in ASFV virulence.

Disease Transmission

In sub-Saharan Africa, cycling of ASFV between soft ticks of the genus Ornithodoros and wild pig populations (warthogs, bush pigs and giant forest hogs) provides a natural reservoir of virus that poses a constant threat to domestic pig populations worldwide. ASF has been reported from most African countries south of the Sahara and more recently from Western and North African countries. In 1957, ASF spread to Portugal and eventually to Spain in 1960 where the disease was endemic until its eradication in 1996. Outbreaks in France (1964, 1967, 1977), Madeira (1965, 1974, 1976), Italy (1967, 1980), Malta (1978), Sardinia (1978 to present), Belgium (1985), and Holland (1986). were controlled by animal quarantine and slaughter, resulting in significant economic losses. Sporadic outbreaks of ASF have also occurred elsewhere in the Caribbean (Dominican Republic in 1978, Haiti in 1979, Cuba in 1977-1980) and South America (Brazil in 1978).

The causative agent, ASFV, is a unique and genetically complex DNA virus. It is the sole member of a newly named Asfarviridae and the only known DNA arbovirus. ASFV shares important properties with poxviruses including genomic structure, terminal cross-links and inverted terminal repeats, a cytoplasmic site of replication, the presence of virion associated enzymes involved in mRNA transcription, and temporally regulated gene expression.

Vaccination

There is no vaccine currently available for ASF. All attempts to vaccinate animals using traditional approaches including infected cell extracts, supernatants of infected pig peripheral blood leukocytes, purified and inactivated virions, infected glutaraldehyde-fixed macrophages, or detergent-treated infected alveolar macrophages failed to induce protective immunity.

ASF vaccine development is significantly hindered by large gaps in our knowledge of the virus and the complex virus-host interactions involved in infection and immunity. However, some important aspects of ASF protective immunity are beginning to emerge.

Recent genetic and biochemical studies have identified and characterized ASF viral genes crucial for aspects of virulence and host range (VHR). These include specific genes with significant functions in viral virulence, macrophage host range, immune evasion, and other significant host range functions. Notably, pigs immunized with live attenuated ASF viruses (LAV) containing engineered deletions of specific VHR genes were protected when challenged with homologous and geographically related heterologous virus strains.

Vaccines in a Control Strategy

Because ASF is a highly infectious disease and no vaccine or treatment is available, "stamping out" or depopulation should be the primary control and eradication strategy.

Research Needs

- Given our poor understanding of ASFV protective immunity, develop and evaluate first generation engineered live attenuated ASF vaccine candidates. An ASF vaccine will have immediate socio-economic benefit for areas of Africa where enzootic disease makes it impossible to raise swine, while at the same time reducing the global threat posed by ASF at the point source.

- Define ASFV strain variation. It is critical to determine how many ASFV strains a vaccine must protect against. Recent cross protection afforded by geographical related strains suggests antigenic variation is not infinite.

- Identify and characterize ASFV protective antigens and host responses. Construction of second generation ASF subunit vaccines will require knowledge of protective antigens and protective host responses.

Rinderpest Virus

Description

Rinderpest is a highly fatal disease of domestic cattle, buffaloes and yaks caused by a morbillivirus of the family Paramyxoviridae. The virus also affects sheep, goats and some breeds of pigs and a large variety of wildlife species, but not all show clinical signs. The virus is related to those that cause measles in people, distemper in dogs, and peste des petits ruminants in sheep and goats. There is evidence that Bos taurus cattle (humpless) are more susceptible to the virus than Bos indicus cattle (Indian, humped).

Rinderpest killed vast numbers of cattle in Europe during the 18th and 19th century and was once known as cattle plague because of its devastating effect. By a combination of slaughter and rigorous quarantine, Rinderpest was eliminated from Europe in the early 1900s.

Morbilliviruses are extremely fragile in the environment; they are sensitive to sunlight, high temperature, low and high pH and chemicals which can destroy their outer lipid-containing envelope.

Nothing is known concerning the molecular factors that determine the virulence or attenuation of different Rinderpest virus strains. Very few sequence changes are needed to alter the virulence of Rinderpest virus; the genome of the cell-culture vaccine strain differs by less than 0.55% from the virulent virus from which it was derived. Selection of a mild form could well be a means whereby the virus evades detection for many years. There is only one serotype and there is no evidence for a persistent or carrier state in recovered animals. After recovery from infection, an animal is immune for life and, consequently, vaccination is a very effective means of controlling this disease.

The Food and Agriculture Organization of the United Nations (FAO) has launched a Global Rinderpest Eradication Programme (GREP) calling for eradication of the virus by the year 2010.

Vaccination campaigns are currently underway in Africa (Pan African Rinderpest Campaign or PARC), West Asia (WAREC) and South Asia (SAREC). Rinderpest has not been reported from West or Central Africa for 10 years and the disease is now confined to two areas of eastern Africa.

Disease Transmission

Natural infection usually occurs via the upper respiratory tract following inhalation of virus-containing aerosols or the oropharynx after ingestion of infected material. Following intranasal and contact challenge, Rinderpest virus can be recovered within 24 hours from the pharyngeal lymph nodes and tonsils. Infectivity is closely associated with mononuclear leukocytes and is not readily detected in plasma and other body fluids. Following primary multiplication in draining lymph nodes, viremia enables the virus to infect lymphoid tissues throughout the body. This increases the viremia, which then

transports the virus to epithelial tissues, especially to those of the alimentary tract where virus-induced cytopathic effects produce the typical lesions.

Rinderpest virus is excreted from epithelial tissues 1-2 days before the appearance of fever or lesions. The amount of excreted virus increases dramatically as the lesions develop and only starts to decline when the immune response becomes detectable some 4-6 days after the start of fever. The virus is usually undetectable by 12-14 days after the start of fever. At the height of virus excretion, 3-6 days after the start of fever, high virus titers can be found in nasal secretions and feces from infected cattle. The copious output of virus explains why the disease can be so contagious despite the fragility of Rinderpest virus. The diarrhea and ocular nasal discharge probably help to increase the transmissibility of the virus by forming infectious aerosols, and by causing greater contamination of the environment.

Vaccination

A live attenuated cell culture vaccine is available that confers lifelong immunity in cattle after a single inoculation. The vaccine was first developed at the former East African Veterinary Research Organisation by serial passage of the virulent bovine rinderpest Kabete 'O' strain. It is safe for use in cattle, buffalo, sheep and goats of all ages and in zoological collections. Due to individual variations in the duration of passive immunity, animals may not be fully immunised until they are vaccinated once at 1-year of age.

Limitations of Current Rinderpest Vaccines

Maternal antibody interference may be a problem in endemic areas but is irrelevant to a naïve population of healthy cattle.

Vaccines in a Control Strategy

Vaccination is at the core of the FAO Rinderpest eradication campaign and extensive emergency vaccination should be implemented should an outbreak occur in the U.S.

Research Needs

- Develop biotherapeutics to enhance ability of vaccine to prevent colonization of target tissues and shedding.

Eastern Equine Encephalitis (EEE) Virus

Description

Eastern equine encephalitis (EEE) virus is a member of the family Togaviridae, genus Alphavirus. It is closely related to Western and Venezuelan equine encephalitis viruses.

EEE is the rarest of the mosquito borne arboviral infections with an average of five human cases reported annually; however, the illness is fatal in 50 percent of cases (compared to 15 percent with West Nile virus), with the highest case-fatality rates observed in young children and adults over 55-years of age. Those who survive the disease may suffer permanent brain damage. Horses are especially vulnerable and the disease is often fatal. Other domestic animals such as donkeys, mules, pigs and calves can also be infected but the severity of the disease is less than in horses.

Disease Transmission

EEE virus has a complex life cycle involving birds and a specific type of mosquito, Culiseta melanura, which lives in marshes and swamps. This mosquito only feeds on birds and not humans and other mammals. In rare cases, the virus can escape from its marsh habitat in other mosquito species that feed on both birds and mammals. These mosquitoes can transmit the virus to people and animals. Horses and people are “accidental” hosts; thus, when bitten by an infected mosquito, people and horses constitute "dead-end" hosts that cause the cycle to stop because the concentration of viral particles in their blood is insufficient to infect another mosquito. Infected people or horses therefore do not represent a source of virus for other people or horses. Birds and wild animals constitute the reservoir species for the virus. Once infected, they carry high concentrations of the virus in their blood before they become immune to the virus. However, their blood contains enough viral particles for a short time, and mosquitoes may become infected during subsequent blood meals on infected animals.

Vaccination

An inactivated EEE vaccine (PE-6) works well against North American strains but does not protect against the less virulent South American strains. The inactivated EEE vaccine is commercially available in various combinations (e.g., VEE, WEE, EEE) but cases continue to occur because of failures to vaccinate foals and to revaccinate older horses. The vaccines require two initial doses given 3-4 weeks apart. The vaccines are safe to

use in all horses, including pregnant mares and foals. It is strongly recommended that all horses be vaccinated at least twice a year, and up to 4 times where EEE is endemic and the vector season is prolonged. Foals from unvaccinated mares may be vaccinated at any age, but they should be revaccinated at 6 months and one year of age to ensure adequate protection.

An experimental EEE vaccine for humans is available to laboratory workers.

Limitations of Current EEE Vaccines

- Two doses or more are required.
- Short duration of immunity.
- Does not prevent the spread of the virus since horses are dead-end hosts.

Vaccines in a Control Strategy

ince wild birds are the main reservoir of the EEE virus, vaccination of horses (the most susceptible animal species) and people will not prevent the spread of infection. The best control strategy at this time is to reduce vector mosquitoes with larvicides and adulticides and to reduce breeding sites. Personal protective measures to reduce mosquito bites are an important approach to prevention. These measures include the use of repellents, appropriate dress, and avoidance of outdoor activity during twilight hours when many mosquitoes are most active.

Research Needs

- Complete agent characterization; e.g., virulence determinants, dose/route associations, vectorial capacity/environmental factors, and the potential for establishment after aerosol exposure (bioterrorism) of humans and horses.
- Understand the natural biology of the EEE; e.g., environmental persistence, overwintering and reintroduction mechanisms.
- Develop vaccines for humans and equids that are effective against aerosol challenge and protects against virulence factors.
- Develop and evaluate biotherapeutics to increase the onset of immunity.

- Develop pen-side virus detection test that can differentiate all major equine encephalitis viruses (e.g., Eastern, Western, and West Nile).

- Discover effective vaccine with appropriate delivery system for wild birds.

Note: Much of the information contained in this report was obtained from the following sources:

Agricultural Research Service (ARS)
Animal and Plant Inspection Services (APHIS)
Center for Disease and Prevention (CDC)
Food and Agriculture Organization (FAO)
Institute for International Cooperation in Animal Biologics (IICAB)
Office of International Epizootics (OIE)
U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID)

Appendixes

Appendix 1

OIE - List A	Animal Agents (Toxins Omitted)
Foot-and-mouth disease	African horse sickness virus
Swine vesicular disease	African swine fever virus
Peste des petits ruminants	Akabane virus
Lumpy skin disease	Avian influenza virus (highly pathogenic)
Bluetongue	Blue tongue virus (exotic)
African horse sickness	Bovine spongiform encephalopathy agent
Classical swine fever	Camel pox virus
Newcastle disease	Classical swine fever virus
Vesicular stomatitis	Cowdria ruminantium (heartwater)
Rinderpest	Foot-and-mouth disease virus
Contagious bovine pleuropneumonia	Goat pox virus
Rift Valley fever	Japanese encephalitis virus
Sheep pox and goat pox	Lumpy skin disease virus
African swine fever	Malignant catarrhal fever virus (exotic)

Highly pathogenic avian influenza
Mycoplasma capricolum/M.F38M.mycoides
Menangle virus

Appendix 2

Capri (contagious caprine pleuropneumonia)
Overlap Agents
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
Mycoplasma mycoides mycoides
Newcastle disease virus (VVND)
Peste des petits ruminants virus
Sheep pox virus
Rinderpest virus
Swine vesicular disease virus
Vesicular stomatitis virus (exotic)