National Program 103

ANIMAL HEALTH

ACCOMPLISHMENT REPORT 2011-2015

United States Department of Agriculture

Research, Education, and Economics

AGRICULTURAL RESEARCH SERVICE

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National Program 103 Animal Health
ACCOMPLISHMENT REPORT 2011-2015

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Executive Summary

Animal health plays a critical role in ensuring a safe and adequate food supply to the United States population and the world. In spite of years of research, challenges remain in animal health with both emerging diseases and diseases that have long been problematic. The mission of the ARS Animal Health National Program is to deliver scientific information and tools to detect, control, and eradicate animal diseases of high national priority. The ultimate goal of the program is to protect and ensure the safety of the Nation's agriculture and food supply through improved disease prevention, protection, mitigation, response, and recovery.

The anticipated products of the animal health research program were captured in an Action Plan at the start of the 5-year national program cycle. Some of the anticipated products in the Action Plan include finding solutions to prevent economic losses from domestic and foreign animal diseases, providing scientific information to maximize on-farm biosecurity practices for naturally or intentionally introduced pathogens, establishing methods to detect, analyze, and respond to new and emerging pathogens, and developing disease prevention tools such as alternatives to antibiotics, vaccines and biotherapeutics.

Research accomplishments in this report are organized under the seven Research Components of the Action Plan. For each component, the report provides the rationale for the research, the anticipated products and impact, followed by examples of accomplishments and contributions to national research priorities.

Component 1: Biodefense Research
The ARS biodefense research activities under Component 1 include research conducted on foreign animal diseases and emerging diseases that pose the greatest threats to the United States. Foreign animal diseases include Foot-and-Mouth Disease (FMD), which is the only agricultural agent classified as a Tier 1 select agent by both the Center for Disease Control and Prevention (CDC) and the Animal and Health Inspection Service (APHIS). Since 2011, the ARS Biodefense Research program has made significant contributions towards the defense of the country against disease incursions as well as food security initiatives worldwide. These contributions include new technologies such as a new "Leaderless" FMD vaccine specifically designed for use in the U.S. National Veterinary Stockpile; the discovery of a new interferon with potential applications in the control of FMD; new safe diagnostic tests to detect Rift Valley Fever virus; diagnostic tests to provide diagnosticians and regulatory agencies with tools to screen imported animals and animal products to prevent introduction of HoBi like viruses into the United States; and a genetically-engineered swine influenza vaccine that confers cross-protection against emerging variant virus strains.

Component 2: Animal Genomics
The research in this component focused on three strategic areas: 1) identify genetic and biological determinants of disease susceptibility; 2) understand host-pathogen interactions, including mechanisms of pathogen immune evasion and host protective immunity; and 3) using genomics tools to develop alternatives to antibiotics to prevent and control priority diseases in

target farm animal populations. Although the application of genomics tools in animal health research is still in its infancy, ARS scientists have made significant progress in several areas, including improving the safety of a recombinant Marek's disease vaccine; identification of a genetic marker associated with reducing susceptibility to Porcine Reproductive and Respiratory Syndrome (PRRS); the identification of regions in the genome of cattle associated with persistent infection of Bovine Viral Diarrhea viruses; the first validated genetic marker test for post-infection control of Ovine Progressive Pneumonia virus; and the development of alternative strategies to enhance gut immunity and mitigate the use of antibiotics using dietary phytonutrients.

Component 3: Zoonotic Diseases

Zoonotic diseases represent one of the leading causes of illness and death in people. The ARS zoonotic disease research program focuses on brucellosis, leptospirosis, and tuberculosis (TB) with the strategic goal of developing countermeasures to prevent disease transmission in domestic livestock and wildlife reservoir hosts. While eradication of TB and Brucellosis remain the goal, outbreaks in domestic animals due to the presence of the organisms in wildlife remains a serious concern. As a result, much of the direction of the research conducted in this area is developing strategies to diagnose and control the diseases in wildlife, including bison and white-tail deer. This includes genetic sequencing of the organisms and studies to better understand the host-pathogen relationship. Since 2011, ARS scientists have made significant contributions towards the control of zoonotic diseases, including improved diagnostics for bovine tuberculosis in cattle and captive cervids; improved vaccination strategies for wildlife against TB and Brucellosis; sequencing the genome of leptospira species to aid in diagnostic and vaccine development; and identified a bacteria that plays an important role in bovine digital dermatitis.

Component 4: Respiratory Diseases

Despite the wide-ranging use of vaccines and antibiotics in animal agriculture, endemic respiratory diseases remain a primary health threat to livestock and poultry. Most respiratory diseases present themselves as disease complexes involving several primary and secondary viral and bacterial pathogens, complicating control and prevention strategies. Importantly, livestock and poultry that develop respiratory diseases have notable decreases in growth performance. ARS scientists have made tremendous strides in understanding host-pathogen interactions, mechanisms of transmission, and the discovery of highly effective diagnostics, vaccines, and alternatives to antibiotics to control respiratory diseases of livestock and poultry. Since 2011, ARS scientists have made significant contributions to control respiratory pathogens, including the development of an effective modified-live Mannheimia haemolytica vaccine; DNA vaccines to effectively control swine influenza viruses that could be considered as a practical alternative to conventional inactivated vaccines; a panviral microarray for detection of swine respiratory viruses in clinical samples; a rapid diagnostic test for pseudorabies surveillance; and a novel recombinant vectored poultry vaccine that is safe and provides effective protection against both Newcastle disease and Infectious Laryngotracheitis.

Component 5: Enteric Diseases

Although many enteric diseases can be prevented through sound biosecurity measures and good management practices, significant scientific gaps remain in our understanding of the gut microbiomes and the role of beneficial microorganisms in controlling and preventing pathogenic

enteric infections. Johne's caused by Mycobacterium avium subspecies paratuberculosis remains a serious disease for dairy farmers. This disease causes significant economic loss and to date accurate diagnostic and control strategies remain elusive. There is also significant gaps in our understanding of polymicrobial infections, enteric disease complexes, and the ecological and host interactions that lead to disease and production losses. Since 2011, ARS scientists have provided key scientific information that has increased our understanding of enteric infections, discovered new potential poultry enteric pathogens, and discovered potential vaccine candidates to prevent Johne's disease.

Component 6: Parasitic Diseases

Parasites represent one of the most diverse groups of organisms that are responsible for hundreds of insidious diseases ranging from enteric diseases to vector-borne hemoparasitic infections. The livestock and poultry industries are severely affected by significant losses in animal production due to lower weight gain, anemia, diarrhea, and death. Of great concern is the increase in anthelminitic resistance by parasites over the years. As loss of these important tools for controlling parasitic infections becomes more common, alternatives must be found to maintain healthy animals and a safe source of food. In addition, controlling hemoparasites is important to the economies of both the beef and dairy cattle industries, but also the U.S. equine industry. Since 2011, ARS scientists have made significant contributions as evidenced by the development of improved diagnostic assays for anaplasmosis and piroplasmosis; developing treatment techniques to clear piroplasma organisms from infected horses; and developing potential vaccine candidates for control of intestinal parasites in cattle as well as identifying some potential genes that may help to naturally reduce the parasite load.

Component 7: Transmissible Spongiform Encephalopathies

Scrapie of sheep, bovine spongiform encephalopathy (BSE) of cattle, chronic wasting disease (CWD) of deer and elk, and variant Creutzfeldt-Jacob disease (vCJD) of humans are all fatal neurodegenerative disorders classified as transmissible spongiform encephalopathies (TSEs). There are no effective treatments or cure, and the origin of TSEs have yet to be determined. Since 2011, ARS scientists have made significant contributions to our understanding of the pathobiology of prion strains, interspecies transmission, and successfully developed new and improved diagnostic methods. These contributions include new diagnostic methods that enable the detection of abnormal prions directly in formalin-fixed paraffin-embedded tissues, and the use of a commercially available test kit to detect abnormal prions from the retina of animals.

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National Program 103 Animal Health

ACCOMPLISHMENT REPORT 2011-2015

Introduction

PURPOSE OF THIS REPORT

The purpose of this report is to review and simultaneously provide an opportunity for customers, stakeholders, and partners to assess the progress made and provide input for future modifications to the National Program or the National Program's research agenda. Retrospective assessments of national programs are conducted every five-years. This report provides accomplishments for the period of 2011-2015. Retrospective assessments allow Agricultural Research Service (ARS) to periodically update the vision and rationale of each National Program and assess the relevancy, effectiveness, and responsiveness of ARS research. Consequently, the report does not attempt to catalogue all the accomplishments of the constituent research projects in the Animal Health National Program (NP 103), but provide examples of the types of achievements obtained by the research in the various areas. Individual scientists or projects are not identified by name in the narrative text; their achievements are described in the context of contributions made to the research priorities identified by animal health stakeholders.

AGRICULTURAL RESEARCH SERVICE

The Agricultural Research Service (ARS) is the principal in-house research agency of the United States Department of Agriculture (USDA). The Agricultural Research Service is one of four agencies in the Research, Education, and Economics (REE) mission and is charged with extending the Nation's scientific knowledge with research projects in agriculture, human nutrition, food safety, natural resources, and the environment. ARS supports more than 2,000 scientists and post docs organized into approximately 750 permanent research projects at over 90 locations across the country and 4 overseas laboratories.

Role

ARS conducts innovative research to find solutions to problems of high National priority that impact the American people on a daily basis. ARS often performs research on high-risk scientific endeavors to make significant breakthroughs in important problem areas. ARS research programs also complement the work of State Colleges and Universities, State Agricultural Experiment Stations, other Federal agencies, and the private sector. Mechanisms for addressing state and local issues are already in place; therefore, activities within ARS focus

on issues having a regional or national scope and where there is a clear federal role. ARS also provides research support to USDA action and regulatory agencies and to a number of other Federal regulatory agencies, including the Departments of State and Defense, the Food and Drug Administration, and the Environmental Protection Agency.

Vision
Leading America towards a better future through agricultural research and information.

Mission
ARS conducts research to develop and transfer solutions to agricultural problems of high national priority and provide information access and dissemination to:

- ensure high-quality, safe food, and other agricultural products
- assess the nutritional needs of Americans
- sustain a competitive agricultural economy
- enhance the natural resource base and the environment, and
- provide economic opportunities for rural citizens, communities, and society as a whole.

National Programs
ARS research is currently organized into 17 National Programs. These national programs provide programmatic direction, coordination, communication, and empowerment to approximately 750 research projects carried out by ARS. The National Programs focus on the relevance, impact, and quality of ARS research.

ANIMAL HEALTH NATIONAL PROGRAM (NP 103)

The mission of the program is to deliver scientific information and tools to detect, control, and eradicate animal diseases of high national priority. Scientists working in program conduct basic and applied research on selected diseases of economic importance to the United States livestock and poultry industries. The goals of the research mission are to produce knowledge and technology to reduce economic losses from infectious, genetic, and metabolic diseases of livestock and poultry.

National Program Leaders
Drs. Cyril G. Gay and Eileen Thacker led the animal health national research program during the period of 2011-2015. Dr. Gay led projects involving viruses and prions and Dr. Thacker projects on bacterial and parasitic pathogens.

Budget
The research budget for the Animal Health Program in 2015 was $68 million. The budget allocated for animal health research provides the funds necessary to maintain the facilities and equipment, operate the facilities and programs, cover salaries, and implement the research.

Scope
The Animal Health National Program currently includes approximately 42 core research projects supported by 101 scientists located at 11 research sites throughout the country.

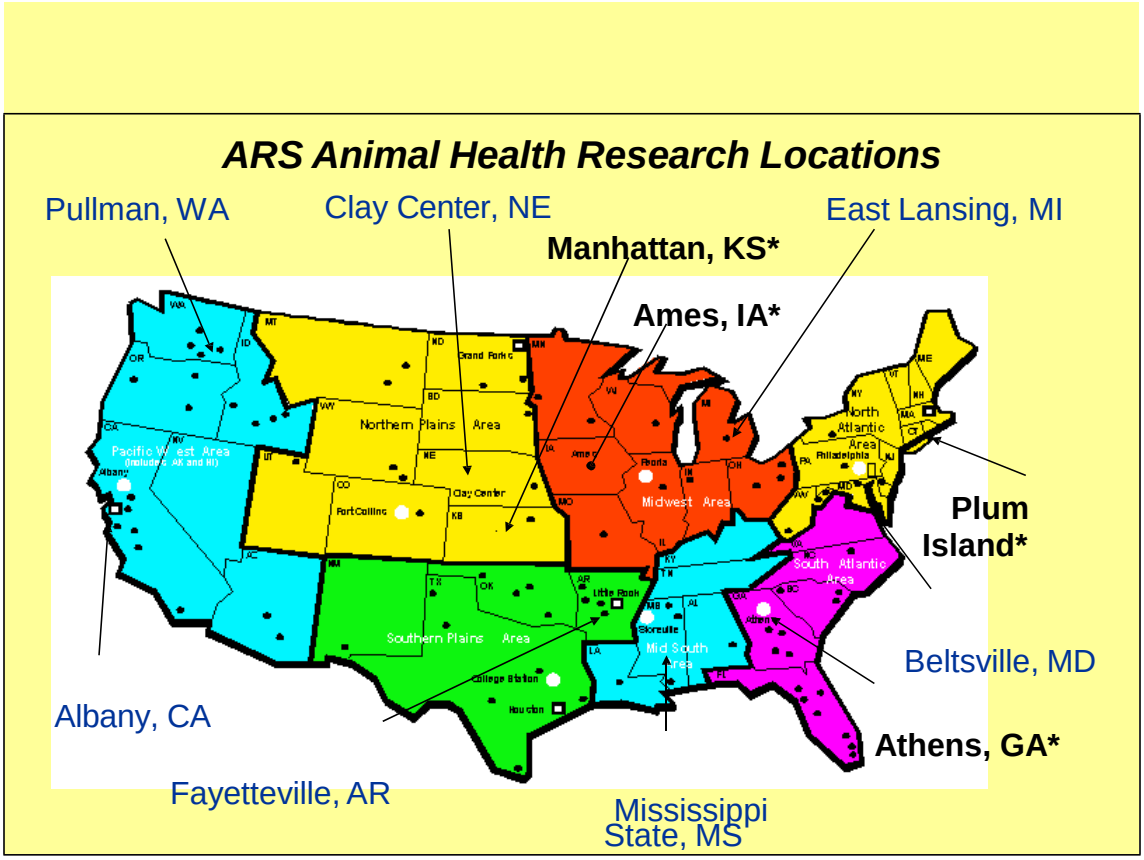


Figure 1: The scientists assigned to National Program 103, Animal Health, are conducting research in 11 different laboratory locations across the United States. * denote locations with select agents and biodefense research.

National Program Cycle

The management and execution of all ARS research programs is organized around the five-year National Program Cycle, consisting of four sequential phases designed to ensure the relevance, quality, and impact of every National Program: 1) Input; 2) Planning; 3) Implementation; and 4) Assessment.

ACTION PLAN

National Programs Leaders prepared an Action Plan for NP 103 at the start of the 5-year national program cycle. This Action Plan addresses the high level goals and actionable strategies associated with the Performance Measures of the ARS Strategic Plan for 2012-2017. Importantly, the Action Plan provides programmatic strategic objectives, specific actions captured under research components, and the anticipated products and impact.

2012-2017 ARS Strategic Plan, Performance Measure 4.2.1: Provide scientific information to protect animals, humans, and property from the negative effects of pests and infectious diseases. Develop and transfer tools to the agricultural community, commercial partners, and government agencies to control or eradicate domestic and exotic diseases and pests that affect animal and human health.

Strategic Objectives
The animal health national program has ten strategic objectives:

1. Establish ARS laboratories into a fluid, highly effective research network, to maximize use of core competencies and resources.
2. Ensure access to specialized high containment facilities to study zoonotic and emerging diseases.
3. Develop an integrated animal and microbial genomics research program.
4. Establish centers of excellence in animal immunology.
5. Launch a biotherapeutic discovery program providing alternatives to animal drugs.
6. Build a technology-driven vaccine and diagnostic discovery research program.
7. Develop core competencies in field epidemiology and predictive biology.
8. Develop internationally recognized World Organization for Animal Health (OIE) expert collaborative research laboratories.
9. Establish best-in-class training center for our nation's veterinarians and scientists.
10. Develop a model technology transfer program to achieve the full impact of our research discoveries.

Research Components
The Animal Health National Program has seven research components:

Component 1: Biodefense Research
Component 2: Animal Genomics
Component 3: Zoonotic Diseases
Component 4: Respiratory Diseases
Component 5: Enteric Diseases
Component 6: Parasitic Diseases
Component 7: Transmissible Spongiform Encephalopathies

Problem Statements
Providing useful information for problem-solving in veterinary medical research often demands an integrated approach where the experimental design may range from knowledge development at the molecular level to clinical trials that will lead to the development of countermeasures for preventing and controlling a disease outbreak in the field. NP 103 utilizes all of these measures to provide the means for the integration of research. For this purpose, select NP 103 projects are aligned under "Major Initiatives." Each major initiative is outlined as Problem Statements under the research components of the Action Plan. Major initiatives draw upon relevant expertise within NP 103, coordinating and integrating that expertise to develop specific useful application of the knowledge. Major initiative projects may also attract federal, university, industry, and international partners. Objectives of major initiative projects are consistent with those of their base projects. When successful, they enhance, rather than detract from, the impact of those base projects on their assigned topics. Because a significant number of projects in the animal health research portfolio focused on the discovery of novel technologies, intellectual property strategies were identified throughout the national program cycle to facilitate technology transfers and investments by the private sector in the development of these technologies.

Anticipated Products

- Finding new solutions to prevent economic losses from domestic and foreign animal diseases in agriculture species.
- Developing methods to help producers adjust to changing farming practices that will allow consumer driven issues to be accommodated without compromising financial viability.
- Providing information that will allow the establishment of on-farm practices to maximize biosecurity from naturally or intentionally introduced pathogens, thus increasing food security, farm productivity, and enhancing trade and exports.
- Establishing methods to detect, analyze, and respond to new and emerging agriculture pathogens.
- Finding solutions to maintain a barrier to pathogens at the domestic-wildlife interface.
- Establishing new detection technologies that will allow better tracking and control of animal pathogens.
- Building an integrated research program to discover genetic variations associated with disease susceptibility and resistance to increase productivity and competitiveness.
- Developing experimental animal disease models that will serve the animal and human health research communities to significantly shorten the timelines for developing breakthrough medicines and disease prevention tools and validate countermeasures.

Impact

During the 5-year period covered in this report, ARS scientists have provided scientific information that has significantly enhanced our knowledge of endemic and foreign animal diseases, including new and emerging diseases such as pandemic H1N1. Scientific information for zoonotic pathogens has been used widely to establish the safety of our food products and support the export of agricultural products. Importantly, a number of tools for detecting and preventing animal disease outbreaks have been discovered and transferred to action and regulatory agencies and the private sector for full development. Specific examples of accomplishments are provided in this report and include the development of a broad range of countermeasures, including diagnostic assays transferred to the Animal and Plant Health Inspection Service (APHIS) and the National Animal Health Laboratory Network (NAHLN) to support surveillance programs, and new vaccine technologies for priority diseases like foot-and-mouth disease (FMD) and Classical Swine Fever (CSF) that have been transferred and are currently being developed by pharmaceutical companies with the support of ARS scientists.

HOW THIS REPORT WAS CONSTRUCTED AND WHAT IT REFLECTS

For the most part, the content of this report is derived from the 2011-2015 annual reports from NP 103 research projects. This report does not include all accomplishments achieved by this national program, rather, only selected accomplishments that illustrate and exemplify the total progress and achievements at the national level.

Accomplishments and their impacts are organized according to the seven Action Plan components listed above. The report first outlines the rationale for the research, followed by the research needs and the anticipated products and impact for each of the components. Then, selected accomplishments are listed as examples of contributions toward the high priority needs identified by stakeholders and described in the NP 103 Action Plan.

Although these research priorities and anticipated products serve to help measure the national program's progress during the last five years, their primary purpose was to provide overarching targets for scientists working in the Animal Health National Program. Importantly, the extensive lists of research priorities in the Action Plan provided direction for acquiring additional resources (extramural funding and/or research collaborations) to build and expand research programs where needed during the course of the 5-year national program cycle.
All of the research projects in NP 103 are listed in Appendix 1; publications in peer-reviewed journals authored by NP 103 scientists are compiled in Appendix 2; patents and technology transfer are listed in Appendix 3; research collaborations are listed in Appendix 4; and a summary of the Stakeholder Retrospective Electronic Survey conducted in 2011 to assess 1) the impact of the Animal Health National Program 2007-2011 and 2) to assess research priorities going forward, is available in Appendix 5.

Component 1: Biodefense Research

Rationale for the research:
The health and well-being of animals in the United States are continuously threatened by exotic animal diseases due to natural events, accidents, or the potential deliberate introduction of an agent into a naïve healthy population of productive animals. These diseases vary in the potential degree of economic loss they cause, their ability to spread, ease of control and ability to eradicate. Many of these diseases are caused by high consequence animal pathogens that are not hindered by international borders and are thus labeled transboundary diseases by the Food and Agriculture Organization (FAO) of the United Nations. Since most of these diseases do not exist in the United States they are also referred to as foreign animal diseases. Of particular concern are emerging animal diseases that challenge our disease surveillance systems and our ability to prepare and respond to disease outbreaks. Some of these diseases are also zoonotic in nature and pose a significant threat to people and have public health implications. Introduction of these diseases in the United States could have devastating social and economic effects not only for the country's agricultural systems but also for a wide range of economic activities, such as the export and trade of agricultural products.

ARS biodefense research activities under Component 1 include research conducted on select agents identified under the Agricultural Bioterrorism Protection Act of 2002. Select agents pose a significant threat to animal or public health or animal products. Research on select agents is regulated by the Animal and Plant Health Inspection Service (APHIS) and/or the Center for Disease Control and Prevention (CDC) and requires high containment laboratories and animal facilities. Outputs under Component 1 are used by Federal and State regulatory agencies for development of policy, surveillance and to mitigate accidental or potential intentional acts of agroterrorism.

The direction provided to scientists assigned to biodefense research purposely targets basic research aimed at increasing our understanding of how disease agents survive outside the host, move between susceptible hosts, infect animals, and how pathogens escape and are shed from the host. To improve our response to disease incursions, the program also allocated significant resources towards the discovery of veterinary countermeasures for the U.S. National Veterinary Stockpile. The research program has successfully established strategic international research collaborations with scientists in countries where foreign animal diseases (many of which are select agents) are endemic. One notable recent accomplishment was the creation of the Global African Swine Fever Research Alliance (GARA - http://www.ars.usda.gov/gara). Partnerships with researchers in other countries contribute to the program by providing access to samples, scientific information, resources, and the ability to test countermeasures in endemic settings.

Stakeholders who completed the 2011 retrospective electronic survey representing the beef and the poultry layer industries ranked foreign animal diseases their 1st priority; the poultry broiler industry their 2nd priority; the dairy industry their 5th priority; and the swine industry their 5th priority. Diseases in Component 1 include Foot-and-Mouth Disease; Influenza; Rift Valley Fever; Classical Swine Fever; African Swine Fever; virulent Newcastle disease; Vesicular Stomatitis; and exotic Bluetongue. In December of 2014, ARS added Porcine Epidemic Diarrhea Virus (PEDV) to its research portfolio; however, since this research just started so

accomplishments are not included in this report.

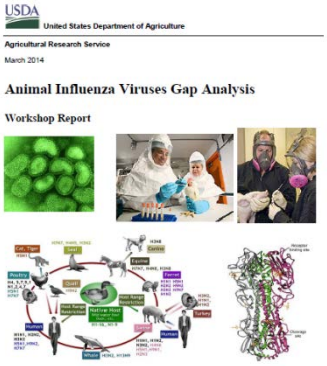
Research needs:
In order to control foreign animal diseases, a wide variety of agent detection platforms need to be developed and validated. Information for designing these platforms requires the use of genomics and proteomics to understand the evolution and genetic variability of disease agents. There is a dearth of knowledge for many priority foreign animal diseases, such as understanding the host range; their primary site of replication; tissue tropism; carrier state; duration and routes of shedding; transmission mechanisms (e.g., vectors, fomites, aerosols); ecology and epidemiology, including wildlife reservoirs. If a disease outbreak should occur in the United States, effective veterinary countermeasures are needed to support suitable control strategies compatible with a short time to recovery to limit the economic impact. There is a need for developing vaccines and biotherapeutics suitable for the U.S. National Veterinary Stockpile. Importantly, integrated approaches for responding to foreign animal disease outbreaks are needed to enhance our capability to regain country disease-free status and retain economic sustainability.

Since no one can predict with certainty the pathogen that will cause the next pandemic, there is a need to continuously isolate, identify, and characterize pathogen(s) associated with new disease complexes of unknown etiologies. The capabilities to rapidly detect, identify, and characterize new and emerging animal pathogens are paramount and a primary goal of the biodefense research program. Scientists need to conduct animal studies in the relevant host to fulfill Koch's postulate and determine the pathogenesis of monovalent and multivalent infections. Once a new agent is isolated there is a need to sequence partial or complete microbial genomes to identify unique sequences for diagnostic discovery and molecular epidemiology research. Research is needed to understand mechanisms of disease, disease transmission, and host range specificity to determine the prevalence and emerging potential of new diseases. Ultimately research is needed to identify mitigation strategies for disease emergence and outbreaks. Establishing strategic international research collaborations is critical to address many of these research needs.

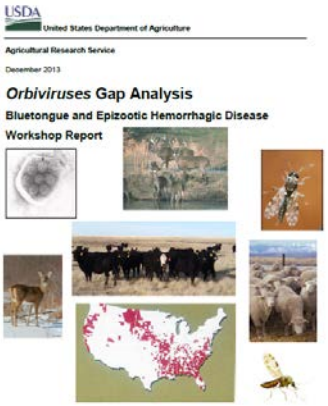
Many of the specific research priorities and anticipated products for Component 1 were derived from gap analyses conducted by worldwide experts in the last five years. These gaps analyses were organized and led by ARS at the request of stakeholders and partners, such as the U.S. National Veterinary Stockpile (NVS) Steering Committee (http://www.aphis.usda.gov), the Global Strategic Alliances for the Coordination of Research on the Major Infectious Diseases of Animals and Zoonoses (http://www.star-idaz.net/), and the United States Animal Health Association (http://www.usaha.org). Some of the results from these gap analyses are confidential due to recommendations made in the reports for stockpiling countermeasures. However, several reports have been amended for public distribution and are available on the following ARS website: http://www.ars.usda.gov/research/programs/programs.htm?np_code=103&docid=17547. Some of these reports have been instrumental in shaping research priorities worldwide. The following three examples illustrate some of the impact resulting from these gap analysis workshops.

An example of gap analysis acquired information was through the Foot-and-Mouth Disease (FMD) Gap Analysis Workshop organized in Buenos Ares, Argentina, August 2010. The report from this workshop (http://go.usa.gov/kCqF) was instrumental in setting the research agenda and activities of the Global FMD Research Alliance (http://www.ars.usda.gov/GFRA). Importantly, the report provided concrete information on the gaps in the scientific information and tools available for controlling FMD, and a list of research priorities for addressing those gaps. This information was critical in guiding funding agencies as well as the organization of additional GFRA workshops in Africa and Asia. In addition, the FMD gap analysis workshop report served as the basis for disseminating critical information in the scientific literature to enable the development of the most promising countermeasures; e.g., Rodriguez L.L. and Gay C.G. 2011. Development of vaccines toward the global control and eradication of foot-and-mouth disease. Expert Rev of Vaccines, 10 (3): 377-387.

A second example of information gained was the Animal Influenza Viruses Gap Analysis Workshop organized in Athens, Georgia, March 2013. The report from this workshop (http://go.usa.gov/KpGP) updated research priorities and contributed to several priority setting workshops organized by international partner organizations, including the OIE/FAO network of expertise on animal influenza (OFFLU - http://www.offlu.net) and the European Food Safety Authority (EFSA - http://www.efsa.europa.eu/en/events/event/150108.htm). The results of the workshop organized by EFSA are expected to lead to future research activities to be financed by the European Commission under Horizon 2020, including support for integrating and enabling international research collaborations worldwide (http://ec.europa.eu/programmes/horizon2020/en/news/major-knowledge-gaps-yet-be-filled-fight-animal-influenza).



The third example is the Orbivirus Gap Analysis Workshop organized in Manhattan, Kansas, May 2013. This workshop was organized in response to USAHA Resolution 16, requesting the USDA and the United States Department of Interior to organize a diverse panel of experts, including industry stakeholders, university and federal researchers, and federal and state regulatory agency representatives to determine research needs and identify and prioritize intervention strategies. The report from this workshop (http://go.usa.gov/BJ5F) was instrumental in identifying research priorities that are likely to have impact, including directing newly appropriated funds to address many gaps and challenges. In addition, the workshop report has served as the source for disseminating critical information to our



stakeholders and partners, including a special issue in the journal Vector-Borne and Zoonotic Diseases dedicated to the results of the workshop gap analysis (Publication pending as to the date of this report).

The reports from these gap analysis workshops and our continuous efforts to engage stakeholders and partners provided the research priorities and anticipated products that now measure the National Program's progress during the last five years in meeting the needs of animal producers, researchers, and action and regulatory agencies.

The following list of anticipated products from the Action Plan are immediately followed by the expected impact of the research and a sampling of relevant accomplishments.

Anticipated Products from the Action Plan:

- Improved ability to predict or anticipate the emergence and introduction of foreign animal diseases.
- Capability to advise federal and state officials on scientific procedures for preventing the introduction of foreign animal diseases.
- Better capability to produce effective products to control and eliminate foreign animal diseases.
- Real-time detection of agents in a wide range of farm matrices.
- Searchable databases of genome and proteome information for major known foreign animal diseases agents.
- Discovery of effective candidate biotherapeutics.
- Discovery of effective candidate vaccines that allow differentiation of infected animals from vaccinated animals (DIVA).
- Viable integrated vector control strategies that minimize losses.
- Identification of new pathogens associated with emerging diseases.
- Establishment of methods to rapidly detect and characterize the etiology of new and emerging diseases.
- Development of predictors of new and emerging disease outbreaks.
- Tools and expertise to mitigate emerging diseases and rapidly implement countermeasures to respond to new disease outbreaks.

Impact:

The research provides improved methods for the prevention and control of select agents, which are considered high consequence pathogens. The research yields scientific information on disease transmission, pathogenesis, and intervention strategies to enable the detection, control and eradication of foreign animal diseases and/or new emerging diseases of humans and animals.

COMPONENT 1: SELECTED ACCOMPLISHMENTS

Problem Statement 1A: Foreign Animal Diseases

A Safe Leaderless Foot-and-Mouth Disease Vaccine Platform with Two Negative Markers for Differentiating Infected from Vaccinated Animals.

ARS scientists at the Plum Island Animal Disease Center identified that the lead sequence, if removed, renders the FMD virus harmless to animals while still leaving it capable of growing in cell culture. This basic scientific information has contributed to our understanding of how the FMD virus amplifies, interacts with an animal host, evades the host defense mechanism, and how different parts of the virus genome function. Importantly, ARS scientists used this information to produce a new "leaderless" FMD vaccine virus. This vaccine is safer than current FMD vaccine technologies, which use naturally occurring (wild-type) virus, because the leaderless attenuated FMD vaccine virus does not cause disease in animals. This is a major milestone in vaccine technologies in that it will enable the safe production of FMD vaccines, eliminating concerns that FMD vaccine viruses might escape from a manufacturing plant and cause an FMD disease outbreak. This will be especially beneficial for FMD-free countries like the United States, providing the capability to rapidly manufacture millions of FMD vaccine doses without fear of vaccine virus escapes. In addition, the leaderless FMD vaccine has been genetically modified to include two negative markers to differentiate it from wild-type virus found in animals during a disease outbreak. A patent has been filed for this new technology, which is currently being developed in partnership with a multinational pharmaceutical company.

Scientific Publication

Sabena Uddowla, Jason Hollister, Juan M. Pacheco, Luis L. Rodriguez and Elizabeth Rieder. A Safe Leaderless Foot-and-Mouth Disease Vaccine Platform with Two Negative Markers for Differentiating Infected from Vaccinated Animals. J. Virol. 2012, 86(21):11675. DOI: 10.1128/JVI.01254-12.

Induction of the Cellular Immune Response to Foot-and-Mouth Disease Virus by Unique Vaccine Targeting.

The host response to FMDV infection has historically been focused on neutralizing (virus blocking) antibody responses. However, the cellular immune response has been much more difficult to assess. ARS scientists at the Plum Island Animal Disease Center, Greenpoint, New York, discovered vaccination strategies to induce the humoral or cellular response separately so it could be determined what the contribution of these different responses were in protecting animals from infection. The experimental FMDV vaccine, vectored by human replication defective adenovirus 5, induces strong humoral immune responses but no cellular immunity. An altered Ad5-FMDV construct induces cellular immune responses and poor humoral immunity. The research showed for the first time that the cellular response to FMDV, in the absence of significant humoral immunity, reduces clinical disease, most notably by blocking virus spread via blood in animals challenged with live virus. This approach reveals the effect of cellular immunity alone and indicates that adding vaccination for cellular immune responses has the potential to improve the performance of the Ad5-FMDV vectored vaccine.

Scientific Publication
Patch J.R., Pedersen L.E., Toka F.N., Moraes M., Grubman M.J., Nielsen M., Jungersen G., Buus S., Golde W.T. 2011. Induction of foot-and-mouth disease virus-specific cytotoxic T cell killing by vaccination. Clin Vaccine Immunol. 2011 Feb; 18(2): 280-8. Epub 2010 Dec 22.

Increased Efficacy of an Adenovirus-vectored Foot-and-Mouth Disease Virus Vaccine Expressing Nonstructural Protein 2B.
ARS scientists at the Plum Island Animal Disease Center, Greenpoint, New York, previously demonstrated that an adenovirus-vectored FMDV serotype A24 vaccine, Ad5-A24, expressed under the control of a cytomegalovirus promoter (CMV) can protect swine and bovines against homologous challenge, but swine vaccinated with an Ad5-vectored FMDV O1 Campos vaccine are only partially protected when challenged 21 days post-vaccination. ARS scientists have now demonstrated that inclusion of the complete coding region of nonstructural protein 2B in the Ad5-A24 vector resulted in improved immune responses in pigs. Interestingly, although a significant antigen specific-CD8+ T cell response was also detected in all vaccinated groups, it was higher in the group receiving the Ad5-vectored FMDV O1 Campos vaccine with the complete 2B coding region. These results indicate that a vector containing 2B improves the efficacy of Ad5 vaccines.

Scientific Publication
Moraes M.P., Segundo F.D., Dias C.C., Pena L., Grubman M.J. 2011. Increased efficacy of an adenovirus-vectored foot-and-mouth disease capsid subunit vaccine expressing nonstructural protein 2B is associated with a specific T cell response. Vaccine. 2011 Nov 28; 29(51): 9431-40. Epub 2011 Oct 24.

Type I Interferon Rapidly Protects Swine against Challenge with Foot-and-Mouth Disease Virus.
Foot-and-mouth disease virus (FMDV) vaccines require approximately seven days to induce protection, but prior to this time vaccinated animals are still susceptible to the disease. Type I interferon (IFN-alpha/beta) is the first line of host defense against viral infection and upon its induction results in the up-regulation of hundreds of IFN-stimulated genes and their products. ARS scientists at the Plum Island Animal Disease Center, Greenpoint, New York, previously demonstrated that intramuscular (IM) inoculation, at one site in the right hind limb, of a replication-defective human adenovirus type 5 (Ad5) vector containing the porcine IFN-alpha gene (Ad5-pIFNalpha) can sterilely protect swine challenged one day later by direct intradermal (ID) needle infection with FMDV serotypes A O1 Campos, Asia-1, as well as A24 Cruzeiro and against A24 Cruzeiro in a direct contact challenge model. To attempt to reduce the protective dose of Ad5-pIFNalpha, ARS scientists inoculated animals IM at four sites in the neck with a lower dose. Eighty percent of animals were sterilely protected as compared to only 33 percent of animals inoculated IM at one site in the right hind limb. These proof-of-concept studies demonstrate the utility of Ad5 delivered type I IFN as a means to rapidly protect swine against FMDV and suggest that various modifications of this approach may enable this strategy to be successfully used, on a practical scale, to treat other FMDV susceptible species.

Scientific Publication
Dias C.C., Moraes M.P., Segundo F.D., de los Santos T., Grubman M.J. 2011. Porcine type I

interferon rapidly protects swine against challenge with multiple serotypes of foot-and-mouth disease virus. J Interferon Cytokine Res. 2011 Feb; 31(2): 227-36. Epub 2010 Sep 28.

The Discovery of a New Interferon and its Potential Application in the Control of FMD.
Foot-and-mouth disease (FMD) is one of the most serious threats to the livestock industry. Despite the availability of vaccines, recent outbreaks in disease-free countries have demonstrated that development of novel FMD control strategies is imperative. ARS scientists at the Plum Island Animal Disease Center reported the identification and characterization of bovine (bo) interferon lambda 3 (IFN-λ3), a member of the type III IFN family. Expression of boIFN-λ3 using a replication-defective human adenovirus type 5 vector (Ad5-boIFN-λ3) yielded a glycosylated secreted protein with antiviral activity against FMD virus (FMDV) and vesicular stomatitis virus in bovine cell culture. Inoculation of cattle with Ad5-boIFN-λ3 induced systemic antiviral activity and up-regulation of IFN stimulated gene expression in multiple tissues susceptible to FMDV infection. The result of these studies also demonstrated that the type III IFN family is conserved in bovines and boIFN-λ3 has potential for further development as a biotherapeutic candidate to inhibit FMDV or other viruses in cattle.

Scientific Publications
Diaz San Segundo, F.C., Weiss, M., Perez-Martín, E., Koster, M.J., Zhu, J., Grubman, M.J., De Los Santos, T.B. 2011. Antiviral activity of bovine type III interferon against foot-and-mouth disease virus. Virology 413 (2011) 283–292

Perez-Martin, E., Weiss, M., Diaz San Segundo, F.C., Pacheco Tobin, J., Arzt, J., Grubman, M.J., De Los Santos, T.B. 2012. Bovine type III interferon significantly delays and reduces the severity of foot-and-mouth disease in cattle. Journal of Virology. 86(8):4477-4487.

Developing Diagnostics to Detect Rift Valley Fever Virus Outside of Containment.
Currently, regional veterinary biosafety level 2 (BSL-2) diagnostic laboratories lack safe, modern, validated diagnostic tests to detect Rift Valley fever virus (RVFV). For RVFV antigen detection, reagents are typically produced at BSL-3Ag or BSL-4 conditions and require inactivation and safety testing for use outside of containment. ARS scientists in Manhattan, Kansas, modified an existing one-step, real-time RT-PCR (rRT-PCR) assay for quick virus detection for use in BSL-2 laboratories. The researchers produced an antiserum against recombinant RVFV-nucleocapsid (N) to develop an immunohistochemical (IHC) assay that was subsequently evaluated on formalin fixed lamb and calf tissues at BSL-2 laboratory conditions. Once validated and approved by national regulatory agencies, these assays can be safely produced and distributed to regional diagnostic laboratories, providing capacity for early detection of RVFV in suspected ruminant samples.

Scientific Publication
Drolet, B.S., Weingartl, H.M., Jiang, J., Neufeld, J. Marszal, P., Lindsay, R., Miller, M.M., Czub, M., Wilson, W.C. 2011. Development and evaluation of one-step rRT-PCR and immunohistochemical methods for detection of Rift Valley fever virus in biosafety level 2 diagnostic laboratories. J. Virol. Meth. 179: 373-382.

Development of a Rift Valley Fever Virus Challenge Model to Evaluate Vaccines in Sheep and Goats.

Rift Valley fever virus (RVFV) is transmitted by mosquitoes and causes severe to fatal disease in ruminants and humans, which can be preventable by vaccination. Ruminants are known to amplify RVFV and are a potential source of infection for humans. Availability of a challenge model is a pre-requisite for vaccine efficacy trials. Several modes of inoculation were tested by ARS scientists at the Arthropod-Borne Animal Diseases Research Unit (ABADRU), Manhattan, Kansas, in collaboration with scientists at the National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, Manitoba, Canada. Differences in development of infections in sheep and goats were observed between animals inoculated with RVFV produced in mosquito cells compared to mammalian cells. Only RVFV produced in mosquito cells led to development of virus in the blood (viremia) in all inoculated animals. The insect cell-produced RVFV appeared to be more infectious with earlier onset of viremia, especially in sheep, and may also more closely represent a field situation. These finding were used to develop a challenge protocol suitable for evaluating the efficacy of RVFV vaccines in sheep and goats.

Scientific Publication

Weingartl, H.M., Nfon, C.K., Miller, M.M., Wilson, W.C. 2014. Development of a Rift Valley fever virus viremia challenge model in sheep and goats. Vaccine. 32:2337–2344. DOI: 10.1016/j.

Proposal for a Unified Nomenclature and Classification System of Newcastle Disease Virus Genotypes.

Virulent Newcastle disease viruses (NDV) are found in most countries of the world. Although the United States has strict rules to prevent entry of the virus, it is important to monitor and characterize viruses that pose a threat to the U.S. poultry industry. ARS scientists at the Southeast Poultry Research Laboratory, Athens, Georgia, have obtained strains of NDV from Mexico, Indonesia, Malaysia, Venezuela, Pakistan, Vietnam, Belize, Dominican Republic, South Africa, Peru and from wild birds in the United States and have sequenced and characterized them genetically. The sequences of key genes have allowed the prediction of the virulence of those viruses. This characterization has led to the identification of viruses of Asian lineages for the first time in the American continent (in Peru and in Venezuela) and to the identification of the expansion of the host range of North American virulent Newcastle disease from cormorants to other wild birds and the discovery of this type of viruses on the East Coast of the United States (Massachusetts, Maine, New Hampshire, and Maryland). The discovery of novel NDV on the American continent provides an opportunity to improve the classification of these viruses. Historically, two systems have been simultaneously used to classify NDV isolates into lineages or genotypes, generating confusion in the nomenclature and discrepancies in the assignment of genetic groups. Based on the extensive characterization of NDV collected worldwide, ARS scientists have proposed a unified nomenclature and a classification system based on objective criteria to separate NDV into genotypes resulting in distinct taxonomic groups. Results revealed that class I viruses comprise a single genotype, while class II contains 15 genetic groups including 10 previously established (I-IX, and XI) and five new genotypes (X, XII, XIII, XIV and XV). Adoption of a unified nomenclature and of objective criteria to classify NDV isolates will facilitate studies on NDV epidemiology, evolution, disease control and diagnostics.

Scientific Publications

Diel D.G., da Silva L.H., Liu H., Wang Z., Miller P.J., Afonso C.L. 2012. Genetic diversity of avian paramyxovirus type 1: Proposal for a unified nomenclature and classification system of Newcastle disease virus genotypes. Infect Genet Evol. 12(8):1770-1779

Diel, D.G., Susta, L., Cardenas, S., Brown, C.C., Miller, P.J., Afonso, C.L. 2012. Complete genome and clinicopathological characterization of a virulent Newcastle disease virus isolate from South America. Journal of Clinical Microbiology. 50:378-387.

Genomic Analysis of a Novel Bluetongue Isolate from an Outbreak in California.

Bluetongue is caused by an insect-transmitted virus, which produces widespread edema and tissue necrosis in domestic and wild ruminants that can be fatal. Bluetongue virus (BTV) serotypes 10, 11, 13, and 17 are typically found throughout the United States, while serotype 2 was previously only detected in the southeastern region. However in 2010, serotype 2 was identified in California for the first time. ARS scientists from the Arthropod-Borne Animal Diseases Research Unit in Manhattan, Kansas, in collaboration with scientists at University of California, Davis, the California Animal Health and Food Safety Laboratory, and APHIS, National Veterinary Services Laboratory, Ames, Iowa, sequenced the isolate and determined that the virus was closely related to strains circulating in the southeast. Additional whole genome sequences of related strains were generated and compared with the novel California isolate. The results of this analysis suggest co-circulation of these viruses in the southeastern United States, and supports the preliminary finding that the western isolate is related to recent southeastern strains. This study further supports the need for an ongoing entomologic and livestock surveillance program for this economically important livestock disease.

Scientific Publication

Natasha N. Gaudreault, Christie E. Mayo, Dane C. Jasperson, Beate M. Crossley, Richard E. Breitmeyer, Donna J. Johnson, Eileen N. Ostlund, N. James MacLachlan, and William C. Wilson. 2014. Whole genome sequencing and phylogenetic analysis of Bluetongue virus serotype 2 strains isolated in the Americas including a novel strain from the western United States. Journal of Veterinary Diagnostic Investigation. 26(4)553–557.

Susceptibility of North American White-tailed Deer and Dorset Sheep to the European Strain of Bluetongue Virus Serotype 8.

Introduction of exotic strains of bluetongue virus (BTV) to the United States is a constant threat to our wildlife and livestock. Currently, only five BTV serotypes are considered domestic; however, since 1999, 10 exotic serotypes have been isolated from U.S. livestock and/or wildlife. Of particular concern for introduction is the European strain of BTV-8 (EU-BTV-8). This virus strain caused unprecedented levels of disease and mortality in livestock all across Northern Europe when introduced in 2006. In the United States, the species most susceptible to BTV are white-tailed deer (WTD) and sheep. To determine the disease risk of our WTD and the most common breed of sheep (Dorset) from an introduction of EU-BTV-8, ARS scientists from the Arthropod-Borne Animal Diseases Research Unit in Manhattan, Kansas, in collaboration with scientists at Colorado State University and APHIS, National Wildlife Research Center, Fort Collins, Colorado, conducted experimental infection studies. Both species were highly susceptible to EU-BTV-8 and infection resulted in significant clinical disease; however, disease

levels were similar to what is seen with our domestic serotypes of BTV. These studies demonstrate that our WTD and sheep herds would be highly susceptible to an outbreak of this European BTV-8 were it to be introduced into the United States, but morbidity and mortality would likely be similar to severe outbreaks with currently circulating serotypes.

Scientific Publication
Drolet, B.S., L.M. Reister, J.O. Mecham, W.C. Wilson, P. Nol, K.C. VerCauteren, T.C. Ruby, P.A. van Rijn, R.A. Bowen. 2013. Experimental infection of white-tailed deer (Odocoileus virginianus) with Northern European bluetongue virus serotype 8. Veterinary Microbiology. 166 (2013) 347–355.

Vector Competence of Culicoides sonorensis Midges to Epizootic Hemorrhagic Disease Virus Serotype 7.
Culicoides sonorensis (Diptera: Ceratopogonidae) is a vector of epizootic hemorrhagic disease virus (EHDV) serotypes 1 and 2 in North America, where these viruses are well-known pathogens of white-tailed deer (WTD) and other wild ruminants. Although historically rare, reports of clinical EHDV infection in cattle have increased in some parts of the world over the past decade. In 2006, an EHDV-7 epizootic in cattle resulted in economic loss for the Israeli dairy industry. White-tailed deer are susceptible to EHDV-7 infection and disease; however, this serotype is exotic to the United States and the susceptibility of C. sonorensis to this cattle-virulent EHDV is not known. ARS scientists at the Arthropod-Borne Animal Diseases Research Unit in Manhattan, Kansas, in collaboration with scientists at the Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, Georgia, examined whether C. sonorensis is susceptible to EHDV-7 infection and would be a competent vector should EHDV - 7 be introduced into the United States. Results of the study showed that midges were susceptible to infection with EHDV-7 and the infected midges were able to transmit the virus to a susceptible WTD. Thus, C. sonorensis should be considered a potential vector of EHDV-7. Together with previous work, this study demonstrates that North America has a susceptible ruminant and vector host for this exotic, cattle-virulent strain of EHDV-7.

Scientific Publication
Ruder, M.G., E. W. Howerth, D.E. Stallknecht, A.B. Allison, D.L. Carter, B.S. Drolet, E. Klement, D.G. Mead. 2012. Vector Competence of Culicoides sonorensis (Diptera: Ceratopogonidae) to Epizootic Hemorrhagic Disease Virus Serotype 7. Parasites and Vectors 5:236-243.

Problem Statement 1B: Emerging Diseases

Genetic Evolution of Novel Reassortant Swine Influenza Viruses with the Capability of Infecting Humans.
Swine influenza A virus normally causes a respiratory disease in swine similar to seasonal flu in humans. However, there is evidence that new strains are emerging with potential pandemic and/or zoonotic potential. In collaboration with NIH scientists, ARS scientists at the National Animal Disease Center (NADC), Ames, Iowa, investigated the genetic evolution of novel reassortant swine influenza A viruses detected in the United States and Canada between 2009-2011 with a focus on H3N2 viruses. Analyses included H3N2 viruses designated A (H3N2)

variant (v) because of their capability to also infect humans as recently discovered in the United States, July 2011. Analyses of samples from twelve human cases revealed that the variant swine-lineage H3N2 viruses contained the pandemic matrix (pM) gene from pandemic H1N1 viruses. The A (H3N2) viruses are distinct from contemporary H3N2 circulating in humans and the flu viruses incorporated in the human seasonal flu vaccine, and hence represents a potential pandemic threat. Monitoring and reporting evolutionary dynamics of gene segments in swine at a detailed level is critical to understand how these novel H3N2 viruses emerged in swine and to assess and predict the potential epidemic and/or pandemic threat of variant influenza viruses pose to humans.

Scientific Publications

Kitikoon, P., Vincent, A.L., Gauger, P.C., Schlink, S.N., Bayles, D.O., Gramer, M.R., Darnell, D., Webby, R.J., Lager, K.M., Swenson, S.L., Klimov, A. 2012. Pathogenicity and transmission in pigs of the novel A(H3N2)v influenza virus isolated from humans and characterization of swine H3N2 viruses isolated in 2010-2011. Journal of Virology. 86(12):6804-6814.

Nelson, M.I., Vincent, A.L., Kitikoon, P., Holmes, E.C., Gramer, M.R. 2012. The evolution of novel reassortant A/H3N2 influenza viruses in North American swine and humans, 2009-2011. Journal of Virology. 86(16):8872-8878.

Detection and Control of a Newly Emerging Pathogen: HoBi like Virus.

HoBi like virus is a newly emerging type of virus distantly related to bovine viral diarrhea virus (BVDV) that has been isolated from cattle in South America, Southeast Asia and Europe. Introduction of HoBi like viruses into North America could result in significant economic loss for cattle producers. The clinical presentation following infection with this type of virus is very similar to that seen following infection with BVDV. Like BVDV, HoBi-like viruses cause immune suppression and can establish life-long persistent infection in cattle. HoBi like viruses have not yet been detected in the United States. ARS scientists at the National Animal Disease Center (NADC), Ames, Iowa, have developed tests to provide diagnosticians and regulatory agencies with tools to screen imported animals and animal products to prevent introduction of HoBi like viruses into the United States. These tests have been transferred to APHIS and provide the means to detect and control an introduction if it were to occur in the United States. Since there are no vaccines available for the prevention of infection of cattle by HoBi like viruses, NADC scientists evaluated the protection afforded by current BVDV vaccines to determine their efficacy against this newly emerging virus. It was found that cattle vaccinated against BVDV would have little or no protection against infection with HoBi like viruses. This suggests that new vaccines, specific for HoBi like viruses, need to be developed to control this emerging pathogen.

Scientific Publications

Bauermann, F.V., Flores, E.F., Ridpath, J.F. 2012. Antigenic relationships between bovine viral diarrhea virus 1 and 2 and HoBi virus: Possible impacts on diagnosis and control. Journal of Veterinary Diagnostic Investigation. 24(2):253-261.

Bauermann, F.V., Harmon, A., Flores, E.F., Falkenberg, S.M., Reecy, J.M., Ridpath, J.F. 2013. In vitro neutralization against HoBi-like viruses by antibodies in serum of cattle immunized with

inactivated or modified live vaccines of bovine viral diarrhea virus 1 and 2. Veterinary Microbiology. 166(1-2):242-245.

Bauermann, F.V., Ridpath, J.F., Weiblen, R., Flores, E.F., 2013. HoBi-like viruses: an emerging group of pestiviruses. J. Vet. Diagnost. Invest. 25:6-15.

Ridpath, J.F., Falkenberg, S.M., Bauermann, F.V., Vanderley, B.L., Do, Y., Flores, E.F., Rodman, D.M., Neill, J.D., 2013. Comparison of acute infection of calves exposed to a high-virulence or low-virulence bovine viral diarrhea virus or a HoBi-like virus. Am. J. Vet. Res. 74:438-442.

Bauermann, F.V., Falkenberg, S.M., Vander Ley, B., Decaro, N., Brodersen, B.W., Harmon, A., Hessman, B., Flores, E.F., Ridpath, J.F., 2014. Generation of calves persistently infected with HoBi-like pestivirus and comparison of methods for detection of these persistent infections. J. Clin. Microbiol. 173:3845-3852.

H7N9 Outbreak in China: Animal Investigations and U.S. Animal Health Preparedness Activities.

On March 29, 2013, the Chinese Center for Disease Control and Prevention completed laboratory confirmation of three human infections with an avian influenza A (H7N9) virus not previously reported in humans. By April 26, reports from the China Ministry of Agriculture indicated that the H7N9 virus had been confirmed in chickens, ducks, pigeons (feral and captive), and environmental samples in four of the eight provinces and in Shanghai municipality, confirming that the source of human infections were poultry markets. The USDA set up a Situational Awareness Coordination Unit with a core team of subject matter experts and other USDA representatives, including the Animal and Plant Health Inspection Service, the Agricultural Research Service, the Food Safety and Inspection Service, and the Foreign Agricultural Service. USDA and CDC worked collaboratively to understand the epidemiology of H7N9 infections among humans and animals in China. To date, there is no evidence of this strain of avian influenza A (H7N9) virus has entered the United States. ARS scientists at the Southeast Poultry Research Laboratory, Athens, Georgia, and the National Animal Disease Center, Ames, Iowa, rapidly conducted animal studies to characterize the virus pathogenicity and transmission properties of this virus in avian and swine species. Results from studies performed on poultry and pigs in ARS high-containment facilities indicated that chickens and quail showed no signs of illness but were shedding avian influenza A (H7N9) virus. Pigs infected with the H7N9 virus on the other hand did not amplify or shed the virus. This information was considered critical to prepare first responders in case this new and emerging virus reached the United States. ARS scientists also rapidly developed new diagnostic tests to ensure the virus could be quickly detected, and completed antigenic mapping studies to help identify virus isolates that could be used to develop a vaccine for poultry if needed.

Scientific Publication

Emergence of Avian Influenza A(H7N9) Virus Causing Severe Human Illness — China, February–April 2013 MMWR / May 10, 2013 / Vol. 62 / No. 18.

Complete Genome Sequences of New Emerging Newcastle Disease Virus Strains Isolated from China.

ARS scientists at Southeast Poultry Research Laboratory in Athens, Georgia, in collaboration with scientists at the OIE Reference Laboratory for Newcastle Disease, China Animal Health and Epidemiology Center, Qingdao, China isolated five virulent Newcastle disease virus (NDV) strains from geese in China during 2010 to 2011. The complete sequences of two NDV strains and the sequences of the envelope glycoprotein genes (F and HN) of three other strains were determined. Phylogenetic analysis classified them into a new genotype, designated as genotype XII, which were genetically distinct from genotype VII, the predominant genotype responsible for most outbreaks of Newcastle disease in China in recent years. This is the first report of complete genome sequences of new emerging genotype XII NDV strains isolated from geese in China. This basic scientific information is critical to ensure current molecular diagnostic tests can detect emerging viruses that may migrate from China and pose a threat to the United States.

Scientific Publication

Liu H, Lv Y, Afonso CL, Ge S, Zheng D, Zhao Y, Wang Z. Complete Genome Sequences of New Emerging Newcastle Disease Virus Strains Isolated from China. Genome Announc. 2013 Jan;1(1). doi:pii: e00129-12. 10.1128/genomeA.00129-12.

Virulence Assessment of Asian Highly Pathogenic Porcine Reproductive and Respiratory Disease Virus (HP-PRRSV).

Highly pathogenic strains of porcine reproductive and respiratory syndrome virus (HP-PRRSV) have resulted in high swine mortality throughout Asia for several years. ARS scientists collaborated with APHIS investigators to characterize a Vietnamese HP-PRRSV isolate sent for analysis at the Foreign Animal Disease Diagnostic Laboratory of the USDA. A Chinese strain and a Vietnamese strain of HP-PRRSV were then derived from full-genome plasmids by ARS scientists at the National Animal Disease Center (NADC). A small portion of the HP-PRRSV genome was distributed to diagnostic laboratories to ensure readily detection of the foreign strain if it should enter the United States. ARS scientists at NADC tested the Asian strains in healthy swine, verifying 100% mortality in 4-wk old piglets and <50% mortality in older swine. The Asian PRRSV strains alone also resulted in an overgrowth of commensal bacteria, contributing to the severe clinical symptoms. Furthermore, these scientists found that an internationally approved vaccine was not protective for HP-PRRSV, and that a new formulation must be developed in order to fully protect U.S. swine. To further understand the severe disease, gene expression profiles of lymph nodes after infection with HP-PRRSV were produced by the ARS scientists, as the lymph nodes contain the immune cells needed for fighting infection. Distinct genes were found that have potential antiviral functions. ARS scientists at the NADC, in collaboration with scientists at the University of Denver, examined the enzymatic activity of a small part of a viral protein called the OTU domain. They discovered that the HP-PRRSV region was 40 times more capable of cleaving specific types of a cellular protein called ubiquitin than that of a U.S. strain of PRRSV, VR-2332, that causes only mild disease in pigs. Ubiquitin has been implicated in the regulation of many cellular processes, including the control of immune responses. The actions of the OTU region of HP-PRRSV may correlate with the increased disease seen and may be used as a target for vaccines or drug design.

Scientific Publications

Metwally, S., Mohamed, F., Faaberg, K., Burrage, T., Prarat, M., Moran, K., Bracht, A., Mayr, G., Berninger, M., Koster, L., To, T.L., Nguyen, V.L., Reising, M., Landgraf, J., Cox, L., Lubroth, J., Carrillo, C., 2010. Pathogenicity and molecular characterization of emerging porcine reproductive and respiratory syndrome virus in Vietnam in 2007. Transbound. Emerg. Dis. 57, 315-329.

Miller, L.C., Fleming, D., Arbogast, A., Bayles, D.O., Guo, B., Lager, K.M., Henningson, J.N., Schlink, S.N., Yang, H.C., Faaberg, K.S., Kehrli, M.E., Jr., 2012. Analysis of the swine tracheobronchial lymph node transcriptomic response to infection with a Chinese highly pathogenic strain of porcine reproductive and respiratory syndrome virus. BMC Vet Res 8, 208.

Guo, B., Lager, K.M., Henningson, J.N., Miller, L.C., Schlink, S.N., Kappes, M.A., Kehrli, M.E., Jr., Brockmeier, S.L., Nicholson, T.L., Yang, H.C., Faaberg, K.S., 2013a. Experimental infection of United States swine with a Chinese highly pathogenic strain of porcine reproductive and respiratory syndrome virus. Virology 435, 372-384.

Deaton M.K., Spear A., Faaberg K.S., Pegan S.D. (2014). The vOTU domain of highly-pathogenic porcine reproductive and respiratory syndrome virus displays a differential substrate preference. Virology; 454-455:247-53.
Scientific Publication

Component 2: Animal Genomics

Rationale for the research:
The last fifty years in animal agriculture has seen tremendous improvements in animal breeds based on quantitative genetics and the selective breeding of farm animals. Most of these successes have been achieved through selection using traditional quantitative genetics tools, but the recent access to animal genomes and genome-enabled tools is having a significant impact on selection programs to improve production traits. However, a key challenge remains the selection of animals with improved health traits. For instance, the genetic control of complex traits like disease resistance has been unreliable when applied to outbred populations under field conditions. The number of genes, the extent of their effect on disease susceptibility, and the interactions between them remain unknown. There are also significant gaps in our understanding of animal immunology, contributed in large part by the dearth of available immunological reagents. In spite of these gaps, significant advances made in mouse and human immunology, including innate defense mechanisms, have contributed significantly to our understanding of animal immunology. Continued efforts to develop immunological reagents along with genome-enabled tools are having a major impact on our understanding of functional-genomics and host-pathogen interactions. We now have molecular tools that are propelling animal health research towards new fields of investigation in transcriptomics, proteomics, and metabolomics. The application of animal genomics research has the potential to revolutionize the speed and scope of problem-solving in animal health.

Because the anticipated products that were put forth five years ago for research in animal genomics and immunology are very broad in scope and remain largely inspirational, the ARS animal health national program focused available resources in three strategic areas: 1) identify genetic and biological determinants of disease susceptibility; 2) understand host-pathogen interactions, including mechanisms of pathogen immune evasion and host protective immunity; and 3) the development of alternatives to antibiotics to prevent and control priority diseases in target farm animal populations. Priority diseases studied under this research component include Marek's disease, Mastitis, Porcine Respiratory and Reproductive Syndrome, Ovine Progressive Pneumonia, and Coccidiosis.

Stakeholders who completed the 2011 retrospective electronic survey ranked cross-cutting issues such as animal genomics and immunology to improve animal health as their 2nd priority. The research in Component 2 focuses on understanding the functional genomics of host-pathogen interactions in livestock and poultry, providing important scientific information that cross-cut the other six Research Components of the NP 103 National Program.

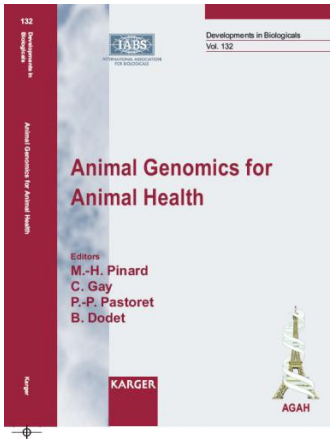
Research Needs:
The significant advances made in the last decade in human biomedical research can be attributed in large part to new breakthroughs in genomics and immunology, including an expanded understanding of the complexities of innate defense mechanisms. However, animal genomics and immunology continues to lag behind its human counterparts. Although the "genomics" revolution is expanding the opportunities for understanding host-pathogen interactions and providing new strategies for developing countermeasures to prevent and treat human diseases, the application of these tools in animal health research is still at the infancy stage.

Research in animal genomics and immunology is needed to understand gene interactions involved in immune cell activation, migration, and host responses. This knowledge will be applied towards the discovery of effective alternatives to antibiotics, including vaccines. A genomics approach will be used to discover naturally expressed antimicrobials. New tools based on protective host proteins will also be developed to modulate innate immune responses to treat infections and/or increase host clearance of pathogens.

The specific research priorities and anticipated products for Component 2 were derived in large part from the outcomes of two international symposiums on "Animal Genomics for Animal Health" organized by ARS in 2007 and 2010. The first symposium was organized in 2007 in collaboration with the World Organization for Animal Health (OIE) and the European Animal Disease Genomics Network of Excellence for Animal Health and Food Safety (EADGENE). One of the major outcomes was the publication of a report capturing the outcomes of the symposium expert panel discussion which included key recommendations and next steps for advancing the integration of genome-enabled technologies in animal health research (Archibald, A., Audonnet, J.C., Babiuk, L., Bishop, S.C., Gay, C.G., McKay, J., Mallard, B., Plastow, G., Pinard van der Laan, MH., Torremorell, M. 2008. Animal genomics for animal health report: critical needs, problems to be solved, potential solutions, and a roadmap for moving forward. Dev Biol (Basel), 132:407-24).

The second symposium was organized in 2010 as a follow up to the first international symposium on animal genomics for animal health http://europepmc.org/articles/PMC3108203. A major outcome was again the publication of a report capturing the outcomes of the symposium expert panel discussion, which identified gaps that hinder the application of genomics in animal health research and specific recommendations for moving the field forward in the next five years (Bishop SC, Lunney JK, Pinard-van der Laan MH, Gay CG. 2011. Report from the second international symposium on animal genomics for animal health: critical needs, challenges and potential solutions. BMC Proc. 2011 Jun 3; 5 Suppl 4:S1).

These two symposiums and the ensuing published reports and recommendations provided research direction, opportunities for research collaborations at the national and international levels, and support from funding agencies such as the European Commission, USDA-NIFA, and the National Pork Board. The reports from these symposiums provided research priorities and anticipated products that were expected from the research and that now serve to help measure the National Program's progress during the last five years in advancing the field of science and the needs of animal producers. The following list of anticipated products is followed by the expected impact of the research and a sampling of relevant accomplishments.



Anticipated Products In Action Plan:

- Identify genetic variations associated with difference in immune cell activation, migration, and host responses to pathogens.
- New methods for preventing and controlling mastitis.
- New biotherapeutic platforms based on protective host proteins to induce the cow's

innate immune response.
- Therapeutics to reduce cell damage during mastitis.
- Innovative approaches using naturally expressed host antimicrobial peptides to increase resistance to prevalent pathogens.
- Basic research information to understand how genetic variations influence the immune response to Marek's disease infection.
- Identification of viral genes responsible for pathogenesis and identification of predictors of virulence shifts.
- Elucidation of viral genes associated with immune evasion mechanisms.
- Characterization of biological pathways that lead to the development of disease.
- Characterization of vaccine-induced determinants of protective immunity.
- Identification of genomic regions or specific structural variations associated with differences in disease-resistance traits.
- Development of enabling tools (immunological reagents, SNP markers and SNP Haplotypes) to propel our understanding of host responses to diseases.
- Identification of the genes mediating resistance from previously determined quantitative trait loci (QTL) associated with susceptibility to diseases of livestock and poultry.
- Biological determinants of innate and adaptive protective immunity will be identified and characterized.
- Highly effective diagnostics, vaccines, and biotherapeutics designed to prevent and control mucosal diseases in targeted animal populations.

Impact:
The development of new genomics and immune-based research approaches provide new synergistic approaches for understanding and mitigating animal diseases that will benefit the animal agriculture industry with the design of effective disease control programs.

COMPONENT 2: SELECTED ACCOMPLISHMENTS

Problem Statement 2A: Oncogenic Diseases of Poultry

Artificial Insertion of Genetic Materials from Reticuloendotheliosis Virus (REV) into Marek's Disease Virus (MDV) Reduces its Pathogenicity.
MDV and REV are both avian viruses that belong to two different groups of oncogenic viruses, MDV is a DNA virus whereas, REV is an RNA virus; both viruses can cause cancer-like disease in chickens. It has been reported that under certain circumstances part of the genetic material from REV know as long terminal repeat (LTR) can be inserted in and be part of the genome of MDV. The effect of REV-LTR insertion into the genome of MDV on the pathogenicity (disease-inducing potential) of MDV is poorly understood. Recently, using a DNA-based technology termed bacterial artificial chromosome (BAC), ARS scientists at the Avian Disease Oncology Research Laboratory, East Lansing, Michigan, were able to artificially insert REV-LTR into a BAC clone of very virulent MDV. The pathogenicity of this BAC clone of MDV with and without REV-LTR was compared in susceptible chickens. The results confirmed those obtained from our previous experiments indicating that BAC clone of MDV containing REV-LTR was less pathogenic than that without LTR or the wild type MDV. This information is

important, as it adds significantly to the knowledge in the area of retroviral gene insertion into large DNA viruses, an important new area of research in the molecular biology of avian tumor viruses. Also, the recombinant virus is now being evaluated as a candidate vaccine for MDV.

Scientific publications

Kim, T., Mays, J.K., Fadly, A.M., Silva, R.F. 2011. Artificially inserting a reticuloendotheliosis virus long terminal repeat into a bacterial artificial chromosome clone of Marek's disease virus (MDV) alters expression of nearby MDV genes. Virus Genes. 42(3):369 376.

Mays, J.K., Silva, R.F., Kim, T., Fadly, A.M. 2012. Insertion of reticuloendotheliosis virus long terminal repeat into a bacterial artificial chromosome clone of a very virulent Marek's disease virus alters its pathogenicity. Avian Pathology. 41(3):259-265.

Improving the Safety of an Efficacious Recombinant (new generation) Marek's Disease Vaccine.

Deletion of the gene responsible for induction of tumors (Meq gene) of Marek's disease virus (MDV) rendered the virus non-oncogenic; in both laboratory and field trials, the new Meq-deleted virus has been shown to be an efficacious vaccine. However, the vaccine caused atrophy (loss of size and weight) of lymphoid organs that are responsible for maintaining a proper immune system in the host. ARS scientists at the Avian Disease Oncology Research Laboratory, East Lansing, Michigan, developed a method to rid the most effective vaccine against Marek's disease from this serious side effect, namely immunosuppression resulting from its negative effects on lymphoid organs. Serial passage of this Meq-deleted Marek's disease vaccine for up to 80 cell culture passages resulted in elimination of its negative effects on lymphoid organs beginning at 40th passage. This development is important, as it will assist vaccine manufacturers to proceed with their plans for commercializing the vaccine.

Scientific publications

Lee, L.F., Heidari, M., Zhang, H., Lupiani, B., Reddy, S.M., Fadly, A.M. 2012. Cell culture attenuation eliminates rMd5deltaMeq-induced bursal and thymic atrophy and renders the mutant virus as an effective and safe vaccine against Marek's disease. Vaccine. 30(34):5151-5158.

Lee, L.F., Kreager, K., Heidari, M., Zhang, H., Lupiani, B., Reddy, S.M., Fadly, A.M. 2013. Properties of a meq-deleted rMd5 Marek's disease vaccine: protection against virulent MDV challenge and induction of lymphoid organ atrophy are simultaneously attenuated by serial passage in vitro. Avian Diseases. 57(2):491-497.

Further Observations on Serotype 2 Marek's Disease Virus-induced Enhancement of Spontaneous Avian Leukosis Virus-like Bursal Lymphomas in ALVA6 Transgenic Chickens.

ARS scientists at the Avian Disease Oncology Research Laboratory, East Lansing, Michigan, have previously shown that certain genetic lines of chickens are susceptible to development of spontaneous tumors termed spontaneous lymphoid leukosis (LL)-like disease, a cancer-like disease that can be detected in chickens in absence of any infection with cancer causing viruses. We have also shown that certain vaccines termed serotype 2 Marek's disease (MD) vaccines that are used to protect chickens from MD can exacerbate spontaneous LL-like disease. Recently, ARS scientists at the Avian Disease Oncology Research Laboratory, East Lansing, Michigan,

reported on the history and effects of MD vaccination on the development of spontaneous LL-like lymphomas during four generations (2009, 2010, 2011 and 2012) of chicken breeders of a line named ALVA6 maintained at the laboratory. Results from this study demonstrated that removal of serotype 2 MDV from MD vaccines used in these breeders eliminated spontaneous LL-like lymphomas at least for 3 consecutive generations. The data also show that in ovo vaccination with serotype 2 MDV did not increase susceptibility of chickens to development of spontaneous LL-like lymphomas. The information should be useful to commercial breeders who may experience such spontaneous LL-like lymphomas in their susceptible lines.

Scientific publication
Weisheng Cao, Jody Mays, Gururaj Kulkarni, John Dunn, Richard M. Fulton & Aly Fadly (2015) Further observations on serotype 2 Marek's disease virus-induced enhancement of spontaneous avian leukosis virus-like bursal lymphomas in ALVA6 transgenic chickens, Avian Pathology, 44:1, 23-27.

Problem Statement 2B: Highly Infectious Diseases of Livestock and Poultry

A Genetic Marker Associated with Reducing Susceptibility to Porcine Reproductive and Respiratory Syndrome (PRRS).
A genetic marker for reduced susceptibility to PRRS, the most economically significant disease in pigs, has been discovered by a research team that includes scientists from ARS in Beltsville, Maryland, Kansas State University and Iowa State University. This project was funded by the USDA National Institute for Food and Agriculture. PRRS affects pigs at all stages of growth and is easily spread. PRRS costs the United States alone an estimated $642 million per year. The PRRS Host Genetics Consortium (PHGC) was established with funds from the U.S. National Pork Board to discover the genetic basis of host resistance or susceptibility to PRRS virus infection. Groups of 200 commercial crossbred pigs were infected with PRRS virus and followed for 42 days; blood samples and body weights were collected for detailed viral load and weight gain “phenotypes.” Ear notches were used to prepare genomic DNA and using Porcine 60K SNP Beadchip generating genotypes on more than 60,000 genetic markers or single nucleotide polymorphisms (SNPs) across the genome for each pig. Using these data, the entire genome of all pigs from the first three PHGC trials were searched to identify chromosomal segments that were common to pigs that had lower virus levels and faster growth after infection. This resulted in the discovery of the genetic marker, called a quantitative trait locus (QTL), on swine chromosome 4 (SSC4) associated with improved growth of pigs that are infected with the PRRS virus. In fact the one Mb region identified on SSC4 influenced both weight gain (WG) and viral load (VL) - 15.7% of the genetic variance for VL and 11.2% for WG. Genomic estimated breeding values (GEBV) for this SSC4 region were perfectly and favorably correlated at -1; i.e., the desired effect when virus decreased, weight increased. Now that scientists have found a genetic region, the next step is to pinpoint the gene and determine whether it shows the same effects for other strains of the PRRS virus. These results could have a major impact in the swine industry by enabling geneticists to develop plans for marker-assisted selection of pigs with improved response to PRRS.

Scientific Publications
Boddicker N., Rowland R., Lunney J.K., Garrick D.J., Reecy J., Dekkers J.C.M. 2011. A major

QTL associated with host response to Porcine Reproductive and Respiratory Syndrome virus challenge. J. Anim. Sci. Epub. 12/28/11.

Lunney J.K., Steibel J.P., Reecy J., Rothschild M., Kerrigan M., Trible B., Rowland R. 2011. Probing genetic control of swine responses to PRRSV infection: Current Progress of the PRRS Host Genetics Consortium. Proceedings of the International Symposium on Animal Genomics for Animal Health (AGAH 2010). BMC Proceedings. 5 Suppl 4:S30.

Lunney J.K, Rowland R. 2011. Understanding Genetic Disease Resistance. National Hog Farmer. Blueprint Immunology 101. Apr. 15, 2011. p.30-42.

Identification of Regions in the Genome of Cattle Associated with Persistent Infection of Bovine Viral Diarrhea Viruses.

Bovine Viral Diarrhea Viruses (BVDV) comprises a diverse group of viruses that cause disease in cattle. BVDV may establish both, transient and persistent lifelong infections depending on the developmental stage of the animal at exposure. Persistently infected cattle represent major losses for the producers because they are more susceptible to infections with other pathogens than normal cattle and they serve as a source of BVDV infections for herd mates. The establishment and maintenance of persistent infections requires a complex interaction between the virus and the host immune system. The interacting host and viral factors are largely unknown. The objective of this study was to identify the regions of the host genome that code for genes that are important in persistent infections. The approach used was to compare the genomes of persistently infected cattle, identified in stockyard populations, to unaffected cattle. This comparison revealed there were sixteen regions in the genome that were different between persistently infected and unaffected cattle. These regions code for genes involved in suppression of the immune system. Identification of these regions lays the groundwork for future studies of the host functions that contribute to the establishment and maintenance of persistent infections. Characterizing these host functions will contribute to the development of means to reduce the impact of persistent infections.

Scientific Publication

Casas E., B. E. Hessman, J. W. Keele, and J. F. Ridpath. 2015. A genome-wide association study for the incidence of persistent bovine diarrhea virus infection in cattle. Anim. Genet. 46:8-15.

First Validated Genetic Marker Test for Post-infection Control of Ovine Progressive Pneumonia Virus.

Ovine progressive pneumonia virus is a small ruminant lentivirus that causes long-term, progressively worsening pneumonia and mastitis in domestic sheep. Some sheep have a genetic predisposition to experience less severe disease from the virus, but there have been no specific genetic tests to predict which sheep these might be. ARS scientists in Pullman, Washington, and Dubois, Idaho, in collaboration with Washington State University, demonstrated that sheep with two copies of a small deletion near the ZNF389 gene were able to control viral replication. This result was observed in multiple sheep flocks under widely differing management and viral load conditions. This is the first validated genetic marker test for post-infection control of ovine progressive pneumonia virus, and it can be used to breed sheep with better ability to control the virus.

Scientific Publication
White, S.N., Mousel, M.R., Reynolds, J.O., Herrmann-Hoesing, L.M., Knowles Jr, D.P. 2014. Variant near ZNF389 is associated with control of ovine lentivirus in multiple flocks of sheep . Animal Genetics. 45(2):297-300.

The U.S. Veterinary Immune Reagent Network.

The U.S. Veterinary Immune Reagent Network (US VIRN, www.vetimm.org) was established to address the lack of immunological reagents specific for livestock and poultry species. Efforts are targeted at swine, ruminants, poultry, equine and aquaculture species. ARS scientists have led the teams for swine and poultry and have successfully developed and characterized bioactive immune proteins, cloned cytokine and chemokine proteins, as well as monoclonal antibodies (mAbs) to these proteins and their receptors and immune cells. These reagents will be used to evaluate swine and poultry immune responses, changes after infections or following vaccination, and give scientists the ability to manipulate these immune proteins, and cell populations to evaluate their roles in protective immunity, immunoregulation, and immunopathology. Recombinant cytokines and chemokines for swine and poultry were cloned and expressed in yeast, purified and shown to be bioactive. All immune proteins developed in this proposal are available to collaborators and have been made commercially available through our U.S. VIRN partner, Kingfisher Biotech, Inc. http://www.kingfisherbiotech.com/. Another goal is to produce mAb reagents that function in different diagnostic platforms. Overall the U.S. VIRN projects are important as a means of identifying new reagents and technologies for veterinary diseases and diagnostic and vaccine discovery research.

Scientific Publications
Hudgens E, Tompkins D, Boyd P, Lunney JK, Horohov D, Baldwin CL. 2011. Expressed gene sequence and bioactivity of the IFNγ-response chemokine CXCL9 of cattle, horses and swine. Vet. Immunol. Immunopathol. 141: 317-21.

Entrican G, Lunney JK. 2011. Veterinary Immunology Committee Toolkit Workshop 2010: Progress and Plans. Vet. Immunol. Immunopathol. Epub.

Boyd P, Hudgens E, Loftus JP, Tompkins D, Wysocki M, Kakach L, LaBresh J, Baldwin CL, Lunney JK. 2010. Expressed gene sequence and bioactivity of the IFNγ-response chemokine CXCL11 of swine and cattle. Vet. Immunol. Immunopathol. 136: 170-5.

Lee, SH, Lillehoj, HS, Park, MS, Baldwin, C, Tompkins, D, Wagner, B, Del Cacho, E, Babu, U, Min, W. 2011. Development and characterization of mouse monoclonal antibodies reactive with chicken CD80. Comp Immunol Microbiol, Infet. Dis. 34:273-279.

Lee SH, Lillehoj HS, Jang SI, Lee KW, Baldwin C, Tompkins D, Wagner B, Del Cacho E, Lillehoj EP, Hong YH. 2011. Development and characterization of mouse monoclonal antibodies reactive with chicken CD83. Vet Immunol Immunopathol. Vet Immunol Immunopathol. 2012. 145; 527– 533.

Lee SH, Lillehoj HS, Jang SI, Baldwin C, Tompkins D, Wagner B, Parcells M, Del Cacho E, Hong YH, Min W, Lillehoj EP. 2011. Development and characterization of mouse monoclonal antibodies reactive with chicken interleukin-2 receptor αlpha chain (CD25). Vet Immunol Immunopathol. 144(3-4):396-404.

Jeong J, Lee C, Yoo J, Koh PO, Kim YH, Chang HH, Choe NH, Lillehoj HS, Min W. 2011. Molecular identification of duck and quail common cytokine receptor γ chain genes. Vet Immunol Immunopathol. 15;140(1-2):159-65.

Problem Statement 2C: Bovine Mastitis

Alternative Strategies for Treating Mastitis in Dairy Cattle.
Mastitis is both the most prevalent infectious disease in dairy herds and the most costly disease for dairy producers. Antibiotics are the mainstay for mastitis treatment and control. Dairy cattle with mastitis receive more antibiotic therapy for its prevention and treatment than for all other dairy cattle diseases combined. Valid concerns by consumers regarding antibiotic usage need to be addressed by research on non-antibiotic alternatives. In preliminary proof-of-concept studies, ARS scientists in Ames, Iowa, showed that vitamin D may be an effective non-antibiotic option for treatment of mastitis. When injected into infected mammary quarters of dairy cows, there was a reduction of mastitis severity. Vitamin D is a simple and natural immune stimulator which, when used in combination with current antibiotics, could become an effective therapy for mastitis. With vitamin D's ability to stimulate the immune system, the time and amount of antibiotics needed to treat mastitis could be reduced. This combination therapy also may be an effective treatment for mastitis infections that are currently resistant to antibiotic treatment alone. The benefits of this therapy could be a reduction in antibiotic residues that may get into the food supply, reduced potential of antibiotic resistance, and an increase in consumer confidence and international trading opportunities.

Scientific Publications
Reinhardt, T.A., Lippolis, J.D., Nonnecke, B.J., Sacco, R.E. 2012. Bovine milk exosome proteome. Journal of Proteomics. 75(5):1486-1492.

Lippolis, J. D., Reinhardt, T. A., Sacco, R. E., Nonnecke, B. J., & Nelson, C. D. 2011. Treatment of an intramammary bacterial infection with 25-hydroxyvitamin D(3). PloS One, 6(10):e25479.

Using a Proteomics Approach, it was Demonstrated that Neutrophil Extracellular Traps (NETS) are a Newly Described and Modifiable Host Mechanism to Battle Mastitis.
Proteomic milk protein expression studies in healthy cows and cows with mastitis provided information important for the dairy food industry and immune function in the mammary gland. Greater than 300 milk proteins associated with host defense were identified and 94 were significantly differentially regulated in Staphylococcus aureus infected glands compared to their

uninfected controls. These differentially regulated host defense proteins were selectively segregated in the milk compartments of whey, exosomes and milk fat globule membranes (MFGM). An example of this segregation of host defense proteins was the partitioning and high concentration of proteins indicative of neutrophil extracellular traps (NETS) formation in the MFGM preparations from Staphylococcus aureus infected milk as compared to exosomes or whey. The practical biological significance of this study is the identification and quantification of the individual components of the NETS functional proteome in an apparent stable complex with MFGM and/or milk fat globules during an intramammary infection. NETS could be functionally relevant in intramammary infection, as it is known that during an infection neutrophils ingest large amounts of milk fat that down regulates many of their traditional immune functions. Thus, the presence of NETs in milk fat provides new insights to mammary immune function and suggests a role for NETs in host mediated clearing of clinical mastitis. These in vivo NETs can now be tested to determine if they retain functional antimicrobial activity when primarily associated with milk fat. Then we can estimate their real world functional relevance during an intramammary infection, which is a key to understanding and treating clinical mastitis in dairy cows. One can envision non-antibiotic methods to enhance NET formation to treat mastitis.

Scientific Publications

Reinhardt TA, Sacco RE, Nonnecke BJ, Lippolis JD. 2013. Bovine milk proteome: quantitative changes in normal milk exosomes, milk fat globule membranes and whey proteomes resulting from Staphylococcus aureus mastitis. J. Proteomics. 82:141-54.

Reinhardt TA, Lippolis JD, Nonnecke BJ, Sacco RE. 2012. Bovine milk exosome proteome. J. Proteomics. 75:1486-92.

Mammary and Whole Body Calcium Homeostasis is Critical to Mammary Health.

Epidemiological studies have shown that hypocalcemia is a focal point in the initiation of a mastitis in the periparturient dairy cow. It was shown that hypocalcemia, especially the subclincal form affects ~ 50% of all transition cows. Furthermore, the speed of involution during the dry period is know to influence new mastitis cases in the periparturient dairy cow. It was demonstrated that calcium may be a key first signal in dry period involution and this has been confirmed by other research groups studing mammary involution. The new knowledge of this calcium mediated involution pathway opens up new opportunities to test non-antibiotic dry cow therapies that use natural compounds to speed dry cow involution via calcium signaling and therefore reduce mastitis in the subsequent lactation.

Scientific Publications

Reinhardt TA, Lippolis JD, McCluskey BJ, Goff JP, Horst RL. 2011. Prevalence of subclinical hypocalcemia in dairy herds. Vet. J. 188:122-4.

Reinhardt TA, Lippolis JD, Sacco RE. 2014. The Ca(2+)/H(+) antiporter TMEM165 expression, localization in the developing, lactating and involuting mammary gland parallels the secretory pathway Ca(2+) ATPase (SPCA1). Biochem. Biophys. Res. Commun. 445:417-21.
Cross BM, Hack A, Reinhardt TA, Rao R. 2013. SPCA2 Regulates Orai1 Trafficking and Store Independent Ca(2+) Entry in a Model of Lactation. PLoS One. 8:e67348.

Determination of Gene Expression during Acute Mastitis in Cattle.
Determination of which genes are changing their expression levels in immune cells in response to an infection has yielded important insights into how the immune system responds to pathogens. ARS researchers in Ames, Iowa, used advanced gene sequencing technologies plus advanced computer programs to study expression changes in important immune genes during the development of an infection in the mammary gland. This was done using the immune cell called the monocyte isolated from milk and blood from animals infected in the mammary gland with Streptococcus uberis. More than 3700 genes in monocytes isolated from milk had changes in their expression levels as a result of the infection. Upregulated genes, due to infection, confirmed some expected genes important to fight infections but more importantly new genes and gene regulators called microRNAs were identified, providing a greater understanding of the molecular and cellular mechanisms in the early stages of an immune cell's response to a mastitis infection. This will help focus research for future management and control of mastitis.

Scientific Publication
Lawless, N., Reinhardt, T. A., Bryan, K., Baker, M., Pesch, B., Zimmerman, D., Zuelke K., Sonstegard T., O'Farrelly C., Lippolis J. D., and Lynn D. J. 2014. MicroRNA Regulation of Bovine Monocyte Inflammatory and Metabolic Networks in an In Vivo Infection Model. G3 4(6):957–971.

Understanding What Makes a Persistent Versus a Transient Mastitis Infection.
Escherichia coli is a leading cause of bacterial mastitis in dairy cattle. Typically this infection is short duration. However, in some cases, E. coli has been shown to cause a long-term infection. The proteins that allow for E. coli infection to become a long-term infection are not known. ARS scientists in Ames, Iowa, studied E. coli that causes short duration or long duration infections. Levels of 1127 proteins were determined. Twenty-eight proteins were found that were associated with either short duration or long duration infections. Of particular interest were proteins that have been shown to be essential for bacterial motility (movement). Bacterial motility assays showed that the strains from the long-term mastitis cases moved significantly greater distances than the strains from the short duration infections. ARS scientists identified key proteins (mobility proteins) associated either with short duration or long duration infections. Understanding the differences between bacteria that cause short duration or long duration infections could lead to targeted interventions to prevent long duration infections.

Scientific Publication
Lippolis, J. D., Brunelle, B. W., Reinhardt, T. A., Sacco, R. E., Nonnecke, B. J., Dogan, B., Simpson K., and Schukken Y. H. 2014. Proteomic analysis reveals protein expression differences in Escherichia coli strains associated with persistent versus transient mastitis. Journal of Proteomics, 108, 373–381.

Problem Statement 2D: Increasing Gut Health and Disease resistance

Development of Alternative Strategies to Enhance Gut Immunity and Mitigate the Use of Antibiotics using Dietary Phytonutrients.

Although widespread use of antibiotic-based growth promoters has improved the efficiency of worldwide poultry production, there is an increasing interest in developing alternative strategies to antibiotics to control infectious diseases in livestock and poultry due to the emergence of drug-resistant pathogens. ARS scientists in Beltsville, Maryland, investigated dietary phytogenics (cinnamon, garlic, and aloe vera) to enhance poultry immunity using avian coccidiosis as a disease model. Phytogenics are a group of natural growth promoters derived from herbs, spices or other plants, and many medicinal foods and herbal products are highly effective in enhancing host defense against microbial infections. ARS scientists previously showed that phytogenics augment host immunity against infectious agents through their ability to alter gene expression. For example, Cinnamaldehyde (CINN) is a constituent of cinnamon that is widely used as a flavoring compound and has been used in some cases to treat human diseases, including inflammatory diseases. CINN has been reported to possess antioxidant, and antimicrobial activities, as well as being able to modulate T cell differentiation. In chickens fed a diet supplemented with CINN, the levels of interleukin (IL)-1 beta, IL-6, IL-15 and interferon-gamma transcripts in intestinal lymphocytes were 2- to 47-fold higher compared with chickens given a non-supplemented diet. Importantly, dietary CINN attenuated Eimeria acervulina and E. maxima-induced bodyweight loss, decreased E. acervulina oocyst shedding, and increased E. tenella-specific antibody responses compared with the non-supplemented control diet.

Scientific Publications

Lee, S.H., Lillehoj, H.S., Jang, S.I., Lee, K.W., Park, M.S., Bravo, D., Lillehoj, E. 2012. Cinnamaldehyde enhances in vitro parameters of immunity and reduces in vivo infection against avian coccidiosis. British Journal of Nutrition. 106:862-869.

Kim, D., Lillehoj, H.S., Lee, S.H., Lillehoj, E., Bravo, D. 2012. Improved resistance to Eimeria acervulina infection in chickens due to dietary supplementation with garlic metabolites. British Journal of Nutrition. p. 1-13.

Yim, D., Kang, S.S., Kim, D.W., Kim, S.H., Lillehoj, H.S., Min, W. 2012. Protective effects of Aloe vera-based diets in Eimeria maxima-infected broiler chickens. Experimental Parasitology. 127:322-325.

Lillehoj, H.S., Lee, S.H., Jang, S.I., Kim, D., Lee, K.W. 2012. Recent progress in understanding host mucosal response to avian coccidiosis and development of alternative strategies to mitigate the use of antibiotics in animal production. Korean Journal of Poultry Science. 38:275-284.

Shirley, M.W., Lillehoj, H.S. 2012. The long view: A selective review of 40 years of coccidiosis research. Avian Pathology. 41(2):111-121.

Complete Amino Acid Sequences of Pheasant and Quail Avian Beta Defensin 2 (AvBD2). Based on previous observation by ARS scientists in Fayetteville, Arkansas, it was shown that it

is possible to identify mature functional peptides in tissue extracts using direct MALDI mass spectrometry. Using this approach, these scientists sought to identify and characterize AvBD2 in avian species where the genome is not yet annotated. Using protein chemistry methods, mass spectrometry for detection and fragmentation, and comparative bioinformatics, ARS scientists determined the amino acid sequences of AvBD2 in both pheasant and quail. The AvBD2 from both species are 36 amino acids long and bear significant homologies to chicken, turkey, duck, and ostrich AvBD2. The defensins are antimicrobial peptides and are an integral part of innate immunity. Because of its abundance, the concentration of AvBD2 in blood may be a useful indicator of pathogen exposure and the status of immunity. Besides immunoassay, mass spectrometry also offers methods that can determine blood levels of AvBD2 in birds.

Scientific Publication

Kannan, L., Liyanage, R., Lay Jr., J.O., Packialakshmi, B., Anthony, N.B., Rath, N.C. 2013. Identification and structural characterization of avian beta-defensin 2 peptides from pheasant and quail. J Proteomics Bioinform. 6:31-37.

Component 3: Zoonotic Diseases

Rationale for the research:
Zoonotic diseases represent one of the leading causes of illness and death in people. By definition, zoonotic diseases encompass all infectious diseases that are transmitted from animals to humans. Zoonotic diseases have a negative impact on commerce, travel, and economies worldwide. In developing countries, zoonotic diseases stand out as the most prevalent and important threat to public health. In industrialized nations, zoonotic diseases remain a concern for human health, but are of particular concern to the agricultural sector as eradication attempts are made. Priority diseases include those that are especially difficult to diagnose and cause substantial morbidity and mortality, resulting in significant economic costs to producers when they persist or reemerge. Because many determinants of zoonotic diseases lie outside the purview of the health sector, agriculture and the animal health community play an important role in controlling these diseases in domestic animals, starting with surveillance systems. Over the years, the USDA has invested significant resources in attempts to eradicate a number of important endemic zoonotic diseases from livestock populations (e.g., brucellosis and tuberculosis). However, their persistence in wildlife reservoirs continues to pose challenges to the eradication process. Moreover, some zoonotic agents have been identified as having the potential to be used for bioterrorism. Effective countermeasures are needed to eliminate zoonotic agents at the source and protect our Nation from these important public health threats.

The ARS zoonotic disease research program focuses on brucellosis, leptospirosis, and tuberculosis (TB) with the strategic goal of developing countermeasures to prevent disease transmission in domestic livestock and wildlife reservoir hosts. Zoonotic diseases that pose a significant threat to the Nation (e.g., avian influenza, Rift Valley fever) and are exotic to the United States are addressed under Component 1: Biodefense Research. Additional zoonotic diseases are also addressed under Component 6 (Babesiosis) and Component 7 (Bovine spongiform encephalopathy).

Stakeholders who completed the 2011 retrospective electronic survey representing the swine and the wildlife industries ranked zoonoses as their 2nd priority; the dairy industry as their 3rd priority; and the beef industry their 4th priority.

Research needs:
Some of the most important gaps in our knowledge about Brucella include the need to analyze the genome to identify unique sequences that can be used for vaccine or diagnostic assay development. This research will help in developing new generation vaccines for control of infections in domestic animals thereby, lowering the incidence of disease and protecting the public and farm workers from Brucella-associated zoonoses. One area that is most important in controlling and potentially eradicating brucellosis is through the identification and characterization of the immune responses in wildlife and domestic livestock to both wild type Brucella species and to the current and new experimental vaccines. Diagnostic and vaccine countermeasures specifically designed for the control and eradication of brucellosis in wildlife needs to be investigated and understood in order to protect domestic livestock.

Leptospirosis remains a serious threat to human and animal health and has become more common as the interface between wildlife, domestic animals, and humans increases due to urban sprawl. Research is needed to characterize spirochete strains associated with field outbreaks, determine how these bacteria interact and elicit host responses during infection, and identify the mechanisms of protective immunity in incidental versus maintenance host infections. Recent progress in genome sequencing of Leptospira borgpetersenii serovar hardjo (the most prevalent serovar of cattle) needs to be extended to other Leptospira species for comparative microbial genomics studies that will help continue to lead to the identification of unique sequences to support diagnostic and vaccine discovery research programs. Diagnostic tools to support molecular epidemiology studies are also needed to understand the ecology of Leptospira species and serovars. In addition, identification of a treponeme as a potential microbe involved in bovine digital dermatitis will assist in developing diagnostic tools and mitigation strategies to control this important cause of lameness in dairy cattle.

Bovine tuberculosis eradication efforts were initiated in the United States in 1917. The eradication program has historically been based on abattoir inspections, testing, and depopulation of infected herds. These efforts have been largely successful. The reactor rate in cattle has been reduced from about 5 percent to currently less than 0.02 percent. Consequently, the incidence of human tuberculosis caused by Mycobacterium bovis has also decreased significantly. However, as the incidence of tuberculosis in the United States declines, Federal and State control programs are facing new challenges. First, there has been a resurgence of bovine tuberculosis in recent years, both in domestic cattle herds and wildlife. This resurgence is due to several factors: the importation of M.bovis infected cattle from Mexico; infections in captive deer and elk herds; the presence of tuberculosis in zoo and wildlife species maintained for exhibition; and most recently, the emergence of tuberculosis in a free-ranging wildlife reservoir (i.e., white-tailed deer) in the United States. The detection of tuberculous cattle and wildlife has serious economic consequences, primarily due to restrictions imposed by regulatory officials on the interstate and international shipment of livestock. As a result, USDA/APHIS requested that ARS redirect its tuberculosis research efforts to examine alternatives to abattoir inspections, and test and slaughter campaigns. Specific needs identified include rapid, specific, and accurate diagnostic tests for cattle and wildlife, and the discovery of highly efficacious vaccines directed at cattle and wildlife to mitigate the transmission of M. bovis in infected herds.

Anticipated Products In Action Plan:

- Comparative genomic analyses of Brucella species to identify unique sequences associated with phenotypic variations in virulence, host range, and persistent infections, and to support diagnostic and vaccine discovery research initiatives.
- Scientific information to increase our understanding of immunologic responses in bison, elk, and feral swine to Brucella species, including mechanisms of persistent infections, host tolerance, and protective immunity.
- The development of a safe and efficacious brucellosis vaccine for bison that can be remotely delivered.
- New vaccine platforms designed to control and eradicate brucellosis in elk.
- New vaccine platforms designed to control and eradicate brucellosis in feral swine.
- New diagnostic platforms with improved sensitivity and specificity profiles to facilitate the diagnosis and epidemiologic trace back of Brucella strains in field outbreaks.

- In vitro disease models for Leptospirosis consisting of host cell cultures leading to molecular characterization of host-bacterial interactions, variations in gene expression, and associated pathogenic mechanisms.
- Characterization of protective immune responses to spirochete antigens in large and small animal disease models.
- Large-scale sequence analysis to characterize the genome of selected spirochetes and identify strain specific regions in various Leptospira strains.
- Genetically altered Leptospira bacteria using in vitro and in vivo studies to establish key links between specific genes and phenotype.
- Discovery of efficacious molecular vaccines to prevent the spread of Leptospirosis in domestic animals and wildlife.
- Identification of microbial immunogens critical for development of protective immunity against tuberculosis.
- Scientific information to increase our understanding of the molecular pathogenesis of M. bovis infections.
- Comparative analyses to understand the variations of host immune responses to natural infections by M. bovis versus vaccination as well as neonatal versus adult cattle responses and differentiate infected versus vaccinated animals.
- Discover improved sensitive and specific diagnostic platforms amenable to the rapid screening of large cattle herds for M. bovis.
- Discover diagnostic platforms to differentiate M. bovis infected versus vaccinated animals.
- Discover effective vaccine platforms to prevent and control M. bovis in cattle and relevant wildlife reservoir hosts.

Impact:
The discovery of new countermeasures specifically designed to prevent and control brucellosis in wildlife has assisted in identifying new sources of infections and in developing new intervention strategies for controlling brucellosis in wildlife. Control of brucellosis in wildlife reduces transmission and safeguards our domestic livestock against infection. Scientists in Ames, Iowa, have been successful in developing assays that enable improved fingerprinting of bacteria and allowing bacterial genotyping. In addition, they have developed new diagnostic assays that better allow rapid diagnosis of infected animals. The scientists have also developed safe and effective vaccination strategies for bison for Brucella abortus. Scientists at Ames, Iowa, have determined that elk do not appear to respond to the current vaccines immunologically. This is an important finding as elk are an important reservoir for Brucella abortus in the Greater Yellowstone region.

Leptospirosis is a challenging bacterium to culture. The scientists in Ames, Iowa, have developed several small animal models which will enable them to begin understanding the virulence factors as well as test efficacy of developed vaccines. The functional genomic analysis of Leptospira strains has enabled the identification of virulence determinants, vaccine discovery research, and new diagnostic platforms for classification of field strains. This research will help in developing new generation vaccines that will help control of maintenance and accidental host infections in domestic animals thereby, lowering the incidence of disease and protecting farm workers from spirochete-associated zoonoses. Additionally, scientists in Ames, Iowa, have been

able to identify new wildlife reservoirs. This knowledge will be used to help educate the public to provide protection against inadvertent transmission from wildlife and the resulting disease. Research on M. bovis in Ames, Iowa, has resulted in improved diagnostic capabilities. Because of the difficulty and inconsistency of the caudal tail fold assay, development of a serological assay was considered important. ARS scientists have worked with industry partners to develop several serological assays that have the potential to improve the accuracy and speed of diagnosing tuberculosis in cattle. In addition, efforts have concentrated on diagnostics and vaccination strategies for control of M. bovis in white-tailed deer, an important reservoir for the disease in parts of the country.

COMPONENT 3: SELECTED ACCOMPLISHMENTS

Problem Statement 3A: Brucellosis

Characterization of Pathogenesis of Brucella Infections in Natural Hosts and Challenge Models for Vaccine Evaluation.
Studies by ARS scientists at the National Animal Disease Center, Ames, Iowa, have compared the susceptibility of various natural hosts (cattle, bison and elk) to infection and abortion after exposure to a virulent strain of Brucella abortus. This work has demonstrated that bison demonstrate higher rates of abortion and infection after exposure than cattle and that elk do not abort when infected in accordance with the standard challenge model for cattle. Work in the project has facilitated understanding of the epidemiology of brucellosis in various hosts, and has assisted in improving the experimental challenge model for vaccine evaluation in elk such that abortion and infection more closely mimics field infection. Additional work has evaluated antibiotic resistance of Brucella, and added to basic knowledge of the intracellular life cycle of Brucella.

Scientific Publications
Olsen SC. Brucellosis in the United States: Role and significance of Wildlife Reservoirs. Vaccine 28(S5): F73-F76.

Olsen SC, Carlson SA. 2015. In vitro bactericidal activity of aminoglycosides, including the next-generation drug plazomicin against Brucella spp. International Journal of Antimicrobial Agents. 45: 76-78.

Olsen SC, Johnson CS. 2011. Comparison of abortion and infection after experimental challenge of pregnant bison and cattle with Brucella abortus strain 2308. Clinical and Vaccine Immunology. 18(12):2075-2078.

Olsen SC, Palmer MV. 2014. Advancement of knowledge of Brucella over the past 50 years. Veterinary Pathology 51: 1076-1089.

Plumb GE, Olsen SC, Buttke D. 2013. Brucellosis: “One Health” challenges and opportunities. OIE Scientific and Technical Review 32: 271-278.

Development of New Brucellosis Vaccines or Vaccine Strategies.
Although essentially eradicated in domestic livestock, the persistence of brucellosis in wildlife reservoirs (bison, elk, feral swine, and reindeer) pose a risk for reintroduction to livestock and human infection. Studies have found species differences in susceptibility to brucellosis with bison being more susceptible to abortion and infection as compared to cattle. During this project cycle, new vaccines or vaccine strategies have been evaluated in domestic livestock and bison, elk, and swine in an effort to develop the most efficacious approach in preventing brucellosis. The project has demonstrated that current brucellosis vaccines are not protective in elk, and are less protective in bison as compared to cattle. The project has demonstrated the safety, immunogenicity, and efficacy of the B. abortus strain RB51 vaccine in bison and found that booster vaccination of calves and yearlings enhances protection against abortion or infection. Although the RB51 vaccine is safe, but not efficacious in elk, studies have evaluated combination of RB51 vaccine with adjuvants in an effort to induce greater protection after vaccination of elk. A new vaccine for domestic and feral swine was demonstrated to be safe and efficacious and can be delivered by parenteral or oral routes. Other vaccine candidates have been evaluated as they become available. Alternative delivery methods including pneumatic and ballistic have been evaluated in bison with data suggesting these delivery strategies are safe and protection similar to that induced by standard parenteral vaccination. Development or improvement in vaccines or vaccine delivery systems will protect the investment in the national Brucellosis Eradication Program and facilitate controlling brucellosis in wildlife reservoir hosts.

Scientific Publications
Ivanov AV, Salmakov KM, Olsen SC, Plumb GE. 2011. A live vaccine from Brucella abortus strain 82 for control of cattle brucellosis in the Russian Federation. Animal Health Research Reviews. 12:113-121.

Olsen SC, McGill J, Sacco R, Hennager S. 2015. Efficacy and immune responses of bison after booster vaccination with Brucella abortus strain RB51. Clinical and Vaccine Immunology (In Press).

Olsen SC. 2013. Recent developments in livestock and wildlife brucellosis vaccination. Brucellosis: recent developments towards “One Health”. OIE Scientific and Technical Review 32: 207-218.

Olsen SC, Hennager SG. 2010. Immune responses and protection against experimental Brucella suis biovar 1 challenge in non-vaccinated or RB51-vaccianted cattle. Clinical and Vaccine Immunology. 17(12): 1891-5.

Olsen SC, Johnson CS. 2012. Immune responses and safety after dart or booster vaccination of bison with Brucella abortus strain RB51. Clinical and Vaccine Immunology. 19(5):642-648.

Olsen SC, Johnson CS. 2012. Efficacy of dart or booster vaccination with strain RB51 in protecting bison against experimental Brucella abortus challenge. Clinical and Vaccine Immunology. 19(6):886-890.

Pires AF, Hoar BR, Sischo WM, Olsen SC. 2011. Serological response to administration of

Brucella abortus strain RB51 vaccine in beef and dairy heifers, using needle-free and standard needle-based injection systems. Bovine Practitioner Journal. 45(2):1-6.

Stoffregen WC, Johnson CS, Olsen SC. 2013. Immunogenicity and safety of a natural rough mutant of Brucella suis as a vaccine for swine. Research in Veterinary Science. 95(2013):251-258.

Problem Statement 3B: Leptospirosis

Characterization of the Pathogenesis of Leptospira and Development of Models for Vaccine Development.

There are numerous species of Leptospira and many are capable of causing disease in both human and animal hosts. Leptospirosis impacts food animal production through reproductive losses associated with acute and chronic infection. The role of both domestic and wildlife species as reservoir hosts incur risk of exposure to both humans and domestic livestock. The project has developed laboratory animal models that replicate acute infection in opportunistic hosts, and chronic infection in reservoirs hosts. These models allow characterization of the pathogenesis of leptospirosis in acute and chronic infection, and may be useful for evaluation of new vaccines. Studies have evaluated outer membrane proteins as potential vaccine antigens. Ongoing studies of vaccines in cattle are characterizing immune responses that induce protection against virulent Leptospirosis strains. Collaborations investigating Leptospira infection and diagnosis in companion (horses) and wildlife (sea lions) species have helped characterize the pathogenesis of disease in these hosts, and allowed comparisons to disease progression in domestic livestock.

Scientific Publications

Alt, D.P., Wilson-Welder, J. Expansion of the in vitro assay for Leptospira potency testing to other serovars: case study with Leptospira Hardjo. Biologicals. 2013 Sep;41(5):323-4

Eshghi, A., Pinne, M., Haake, D.A., Zuerner, R.L., Frank, A.T., Cameron, C.E. 2012. Methylation and in vivo expression of the surface-exposed Leptospira interrogans outer-membrane protein OmpL32. Microbiology. 158(Pt. 3):622-635.

Prager, K. C., Alt, D.P., Buhnerkempe, M.G., Greig, D.J. Galloway R.L., Wu, Q., Gulland, F.M.D., Lloyd-Smith, J.O. Antibiotic efficacy in eliminating leptospiruria in California sea lions (Zalophus californianus) stranding with leptospirosis" Accepted for publication by Aquatic Mammals.

Polle, F., Storey, E., Eades, S., Alt, D., Hornsby, R., Zuerner, R., Carter, R. Role of Intraocular Leptospira Infections in the Pathogenesis of Equine Recurrent Uveitis in the Southern United States. J of Equine Vet Sci. 34 1300–1306. 2014.

Prager, K.C., Greig, D.J., Alt, D.P., Galloway, R.L., Hornsby, R.L., Palmer, L.J., Soper, J., Wu, Q., Zuerner, R.L., Gulland, F.M., Lloyd-Smith, J.O. 2013. Asymptomatic and chronic carriage of Leptospira interrogans serovar Pomona in California sea lions (Zalophus californianus). Veterinary Microbiology. 164(1-2):177-183.

Stokes., W., Srinivas, G., McFarland, R., Kulpa-Eddy, J., Casey, W., Walker, A., Draayer, H., Sebring, R., Brown, K., Balks, E., Stirling, C., Klaasen, E., Hill, R., Rippke, B., Ruby, K., Alt, D., Mukhopadhyay, S., Kojima, H., Johnson, N., Rinckel, L., Doelling, V., Jones, B.. Report on the international workshop on alternative methods for Leptospira vaccine potency testing: state of the science and the way forward. Biologicals. 2013 Sep;41(5):279-94.

Wu, Q., Prager, K.C., Goldstein, T., Alt, D.P., Galloway, R.L., Zuerner, R.L., Lloyd-Smith, J.O., Schwacke, L. Development of a real-time PCR for the detection of pathogenic Leptospira spp. in California sea lions. Dis Aquat Organ. 110:165-72. 2014.

Zuerner, R.L., Alt, D.P., Palmer, M.V. 2011. Development of chronic and acute Golden Syrian Hamster infection models with Leptospira borgpetersenii serovar Hardjo. Veterinary Pathology. 49(2):403-411.

Zuerner, R.L., Alt, D.P., Palmer, M.V., Thacker, T.C., Olsen, S.C. 2011. A Leptospira borgpetersenii serovar Hardjo vaccine induces a Th1 response, activates NK cells, and reduces renal colonization. Clinical and Vaccine Immunology. 18(4):684-691.

Characterization of the Etiology of Bovine Digital Dermatitis.

Bovine digital dermatitis (DD) has emerged as a significant cause of lameness worldwide within the dairy industry, impacting reproduction, milk production and general animal welfare. This disease process has also been observed in beef cattle on feed, and is similar to an infectious lameness described in sheep. Preliminary investigations into a lameness observed in wild elk have also indicated similarities to DD. The disease in cattle likely results from colonization by and interaction within a mixed population of microbes. Among these microbes, treponemes are consistently observed, with Treponema phagedenis-like organisms often the most abundant. The project characterized a treponeme isolate obtained from a case of DD by biochemical methods, produced a draft genomic sequence, and demonstrated it is identical to a treponeme isolated from humans. Additional work to isolate aerobic and anaerobic bacterial isolates from DD lesions continues. This work will allow identification of potential diagnostic and vaccine targets in treponemes, characterization of the role of various bacteria in DD lesions, assist in understanding how DD lesions develop, and lead to development of intervention strategies.

Scientific Publication

Wilson-Welder, J.H., Elliott, M.K., Zuerner, R.L., Bayles, D.O., Alt, D.P., Stanton, T.B. 2013. Biochemical and molecular characterization of Treponema phagedenis-like spirochetes isolated from a bovine digital dermatitis lesion. BMC Microbiology. 13:280.

Problem Statement 3C: Tuberculosis

USDA Licensing and Approval of New Tuberculosis (TB) Diagnostic Tests for Cattle and Deer.

The USDA bovine tuberculosis eradication campaign began in 1917. The cornerstone of the eradication effort has been TB testing of cattle using the tuberculin skin test (TST). Although the TST has been instrumental in greatly decreasing the prevalence of bovine tuberculosis in the United States, novel testing methods are needed to overcome current obstacles to eradication.

Until recently, only one new test for bovine tuberculosis had been licensed and approved in nearly a century. The Bovigam assay was licensed and approved in 2000; the result of collaborations between industry (CSL, Prionics), ARS and APHIS over a period of many years. More recently, in 2013 a rapid, serum-based assay (IDEXX M. bovis ELISA) was licensed by USDA. Licensing of the IDEXX M. bovis ELISA represents the culmination of years of cooperative research by ARS, APHIS and IDEXX Laboratories. Although the ELISA is not sensitive enough to replace current testing regimes, the ELISA identifies animals potentially misdiagnosed as non-infected by the TST and Bovigam assay. It also represents a more rapid and less laborious means of whole herd testing.

TB testing of farmed deer requires at least 2 animal handling events. Each handling event risks injury to frightened deer and personnel. Moreover, TB tests currently used in deer require a minimum of three days for results. A rapid TB test requiring a single handling event would significantly reduce risk to animals and personnel, as well as decrease costs through decreased labor and decreased loss of production. ARS researchers, in cooperation with international collaborators, industry (Chembio Diagnostics Inc), and APHIS developed and validated a new rapid, blood-based test for use in deer. Chembio's DPP CervidTB test was licensed and approved in 2013, requires a single handling event and results can be obtained in minutes rather than days. Since approval, over 23,000 deer have been tested. Current testing rates of over 1000 deer/month are outpacing production and delivery by the manufacturer.

Scientific Publications

Waters W.R., Stevens G.E., Schoenbaum M.A., Orloski K.A., Robbe-Austerman S., Harris N.B., Hall S.M., et al. 2011. Bovine tuberculosis in a Nebraska herd of farmed elk and fallow deer: a failure of the tuberculin skin test and opportunitities for serodiagnosis. Vet Med Int. 2011 Apr 14;2011:953985.

MV Palmer, DL Whipple, JB Payuer, CA Bolin. Use of the intradermal tuberculin test in a herd of captive elk (Cervus elaphus nelsoni) naturally infected with Mycobacterium bovis. Journal of Veterinary Diagnostic Investigation. 2011;23:363-366.

WR Waters, BM Buddle, HM Vordermeier, E Gormley, MV Palmer, TC Thacker, JP Bannantine, JR Stable, R Linscott, E Martel, F Milian, W Foshaug, and JC Lawrence. Development and evaluation of an enzyme-linked immunosorbent assay for use in the detection of bovine tuberculosis in cattle. Clinical and Vaccine Immunology 2011; 18:1882-1888.

WR Waters, TC Thacker, BJ Nonnecke, MV Palmer, I Schiller, B Oesch, HM Vordermeier, E. Silva, DM Estes. Evaluation of gamma interferon induced protein 10 responses for detection of cattle infected with Mycobacterium bovis: comparisons to IFN-gamma responses. Clinical and Vaccine Immunology. 2012;19(3):346-351.

KP Lyashchenko, R Greenwald, J Esfandiari, DJ O'Brien, SM Schmitt, MV Palmer, WR Waters. Rapid detection of serum antibody by dual-path platform VetTB assay in white-tailed deer infected with Mycobacterium bovis. Clinical and Vaccine Immunology 2013 Jun;20(6):907-911.

KE Bass, BJ Nonnecke, MV Palmer, TC Thacker, R Hardegger, B Schroeder, AJ Raeber, WR Waters. Clinical and diagnostic developments of a gamma interferon release assay for use in bovine tuberculosis control programs. Clinical and Vaccine Immunology 2013 Dec;20(12):1827-1835.

Development of a Vaccine to Control Bovine Tuberculosis in Wild White-Tailed Deer.
In 1994, a hunter-harvested, wild white-tailed deer in Michigan, was diagnosed with tuberculosis due to Mycobacterium bovis. Subsequent surveys in the region identified a focus of M. bovis infection in free-ranging white-tailed deer in northeast Michigan. This represented the first known reservoir of M. bovis in wildlife in the United States and a serious impediment to the ongoing effort to eradicate bovine tuberculosis from U.S. cattle. Over 62 cattle herds in Michigan have been diagnosed with tuberculosis since the discovery of tuberculosis in wild deer, presumably from direct or indirect contact with infected deer. Surveillance and control measures, including decreasing the deer population through increased hunting, have been in place for over 15 years and a significant reduction in apparent prevalence of tuberculosis in deer has been achieved. However, hunter support is waning and public resentment of control measures is growing. A control measure that could be applied to specific areas of sustained high disease prevalence is vaccination of deer. ARS scientists have shown that both parenteral and oral vaccination of deer with M. bovis Bacille Calmette Guerin (BCG) decreases disease severity and potentially decreases disease transmission. ARS scientists are working with APHIS Wildlife Services scientists in the development of an oral bait vaccine to be used to vaccinate wild deer in northeast Michigan.

In addition to being efficacious, a vaccine must also be safe. Safety is of particular concern in terms of the animal being vaccinated as well as humans that may consume a vaccinated animal. People often consume hunter-killed deer and although disease is not likely to result from human consumption of BCG, false positive results on the tuberculin skin test could interfere with public health monitoring for human tuberculosis. ARS scientists showed that BCG persists up to 12 months in lymphoid tissues of vaccinated deer. Importantly, BCG has never been isolated from those tissues commonly consumed by hunters (i.e. muscle).

Scientific Publications
MV Palmer, TC Thacker, WR Waters, S Robbe-Austerman, and F.E. Aldwell. Persistence of Mycobacterium bovis bacillus Calmette-Guerin (BCG) Danish in white-tailed deer (Odocoileus virginianus) vaccinated with a lipid-formulated oral vaccine. Transboundary and Emerging Diseases 2014; 61:266-272.

MV Palmer, MR Stafne, WR Waters, TC Thacker, GE Phillips. Testing a molasses-based bait for oral vaccination of white-tailed deer (Odocoileus virginianus) against Mycobacterium bovis. European Journal of Wildlife Research 2014; 60:265-270.

MV Palmer, TC Thacker, WR Waters, S Robbe-Austerman. Oral vaccination of white-tailed deer (Odocoileus virginianus) with Mycobacterium bovis Bacillus Calmette-Guerin (BCG). PLoS One 2014; 9:e97031.

Component 4: Respiratory Diseases

Rationale for the research:
In spite of decades of control measures using antibiotics and vaccines, endemic respiratory diseases remain primary health threats to livestock and poultry. The cost of respiratory disease is significant and disease outbreaks often determine the difference between profit and loss for a producer. Most respiratory diseases present themselves as disease complexes involving several primary and secondary viral and bacterial pathogens, complicating control and prevention strategies. The most challenging aspect of dealing with respiratory disease is recognizing that clinical or overt disease is only the tip of the iceberg. The cost goes far beyond the treatment of sick animals and the cost of dead animals. The vast majority of the economic impact is actually due to the hidden cost of sub-clinical disease where animals are infected but show no apparent disease symptoms. Livestock and poultry that develop respiratory diseases have notable decreases in growth performance. Even with livestock and poultry being vaccinated against many of the most common pathogens today, respiratory lesions are still prevalent at slaughter and their impact on weight gain and carcass quality is significant. Important scientific gaps remain in our understanding of respiratory pathogen complexes and the ecological and host interactions that lead to disease and production losses independent of host species. With the current emphasis on reduced usage of antibiotics in livestock and poultry operations, new research approaches are needed to design effective prevention and control programs that will facilitate proper planning, careful attention to livestock and poultry health management, and the discovery of effective countermeasures.

Stakeholders who completed the 2011 retrospective electronic survey representing the beef industry ranked respiratory diseases as their 2nd priority and the sheep industry ranked respiratory diseases such as malignant catarrhal fever (MCF) as their 5th priority. The swine industry ranked porcine reproductive and respiratory syndrome virus (PRRSV) as their 1st priority, preventative health management (innate immunity and alternatives to growth promotants and antibiotics) as their 2nd priority, re-emerging diseases such as swine influenza virus (SIV) and porcine circovirus type 2 (PCV2) were their 3rd priority, and the porcine respiratory disease complex (PRDC) their 4th priority. The poultry broiler industry ranked respiratory disease their 3rd priority and characterized the following respiratory pathogens as priority diseases: infectious bronchitis, infectious laryngotracheitis, turkey rhinotracheitis (TRT) and associated swollen head syndrome (SHS) of chickens (avian pneumovirus), colibacillosis (Escherichia coli), fowl chorea (Pasteurella multocida), turkey coryza (Bordetella avium), Ornithobacterium rhinotracheale (ORT) of turkeys, and Mycoplasmosis (Mycoplasma gallisepticum and Mycoplasma synoviae).

Because of the sheer number of pathogens involved in respiratory diseases, and the ability of many pathogens to cross the species barrier, ARS concentrated their available resources on priority respiratory pathogens associated with the bovine, ovine, porcine, and poultry respiratory disease complexes. Emphasis was given to the design of experimental animal disease models to test newly discovered technologies and countermeasures, with the eventual goal of validating them under field conditions through strategic partnership with industry.

Research needs:
Research needs identified include identifying and understanding the mechanisms of disease transmission of respiratory pathogens in beef production systems as well as the host responses to respiratory pathogens, including mechanisms of immune evasion and protective immunity. Also recognized as a critical need were epidemiological studies to identify reservoirs of priority respiratory pathogens.

The transmission of malignant catarrhal fever to bison in western grazing lands is a concern to the sheep industry. Two of the most important gaps in the understanding of the transmission between the two species include the pathogenesis and immune response in sheep and bison associated with infection. Research in this area will help develop intervention strategies to minimize the risk of the sheep spreading the devastating disease to bison.

Respiratory disease in swine is one of the most serious and costly concerns to the industry. Research is needed to elucidate the pathogenesis of monovalent and polymicrobial infections of swine respiratory pathogens. Pathogens included in the swine respiratory research included porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), *Bordetella bronchiseptica*, and *Haemophilus parasuis*. The mechanisms by which swine respiratory pathogens caused disease due to changes in gene expression of both the porcine host and the bacterial respiratory pathogens during the infectious process are important in developing effective intervention strategies for polymicrobial infections. In addition, research to understand the host responses to respiratory pathogens, including mechanisms of immune evasion and protective immunity are needed.

Research gaps were recognized in the elucidation of the mechanisms of disease transmission of respiratory pathogens in relevant poultry production systems including the interaction and pathogenesis of polymicrobial interactions. Studies are needed to investigate the host response to respiratory pathogens, including mechanisms of immune evasion and protective immunity. In addition, novel vaccine candidates need to be developed and evaluated for a number of pathogens. Improved diagnostic capabilities to enable rapid differential diagnosis of respiratory pathogens on poultry farms are also lacking for many of the common poultry respiratory diseases.

Anticipated Products in Action Plan:
- Develop drug and vaccine delivery systems that target the swine, avian, and ruminant respiratory tract.
- Discover and evaluate alternatives to antibiotics for preventing and treating respiratory diseases of all domestic livestock species.
- Discover diagnostic platforms that can be used to develop on-site tests for respiratory pathogens.
- Discover highly effective vaccines that induce targeted immune responses to prevent colonization of the respiratory tract and prevent shedding and disease transmission in swine and poultry
- Define pathogen interactions that lead to polymicrobial infections and respiratory disease complexes in swine and poultry.
- Characterize the changes in gene expression underlying porcine cellular responses to

infection with respiratory pathogens.
- Characterize global changes in gene expression of porcine bacterial pathogens in response to respiratory infection.
- Define determinants of virulence and characterize mechanisms of infection of respiratory pathogens of cattle, swine, sheep, and poultry.
- Perform genomic and proteomic analysis of ovine and avian respiratory pathogens to determine changes in gene expression during the infection process.
- Identification of microbial genetic variations associated with differences in virulence and disease transmission in sheep, swine, and poultry.
- Characterize mechanisms of immune evasion and protective immunity of swine and poultry pathogens.
- Define the characteristics of aerosol spread for priority respiratory pathogens in relevant poultry production systems.
- Discover differential diagnostics platforms that can be used to develop flock-side tests.

Impact:
The anticipated impact of the research included the identification of disease pathogen reservoirs, understanding pathogen transmission, and the discovery and technology transfer of highly effective diagnostics, vaccines, and biotherapeutics to control and potentially eradicate respiratory diseases from livestock and poultry.

The overall impact of the research to control and prevent endemic respiratory diseases benefits the cattle, swine, sheep, and poultry industries. Improving our understanding of the disease pathogenesis and the discovery and technology transfer of new and improved control measures, including vaccines, may ultimately help control and/or eradicate priority respiratory diseases from livestock and poultry production. The development of effective vaccines may also play a key role in reducing the use of antibiotics in animal production. The overall goal of these projects was to provide scientific information and tools that enable the U.S. livestock and poultry industries to remain competitive and profitable.

COMPONENT 4: SELECTED ACCOMPLISHMENTS

Problem Statement 4A: Ruminant Respiratory Diseases

Complete Genomic Sequences of Mannheimia haemolytica Serotypes A1, A2, and A6 to Support Development of Novel Countermeasures against Bovine Respiratory Disease.
Mannheimia haemolytica is a component of the normal nasopharyngeal bacterial flora of healthy cattle as well as a serious lung pathogen in disease. During periods of stress and/or concurrent respiratory viral infection this organism in particular flourishes in the nasopharynx to become predominant, yielding bacterial loads orders of magnitude above their normal levels. It is likely not mere coincidence that these same stressful periods also exhibit the greatest incidence of lung disease, pneumonic pasteurellosis, commonly referred to as shipping fever. Nasopharyngeal carriage of Mannheimia is most commonly associated with serotypeA2 with generally lesser carriage of serotypes A1 and A6. Under stressful conditions it is serotypes A1 and A6 that respond with relatively massive proliferation, and it is these two serotypes that are most commonly associated with disease. This specificity of response suggests that a receptor-ligand

interaction is likely mediating the response between host and bacterium. To understand the genetic basis of this host-bacterium interaction under stress, and to seek specific targets for future vaccine development, ARS scientists at the Meat Animal Research Center and the National Animal Disease Center, Ames, Iowa, sequenced several representative isolates of M. haemolytica serotypes A1, A2, and A6. Comparative analysis of those isolates identified a number of putative adhesions, transporters, and outer-membrane proteins in both serotypes A1 and A6, which are very divergent or absent in serotype A2. Identification of these gene targets, and potentially others, is likely to lead to new control methods for Mannheimia in cattle respiratory disease.

Scientific Publications

Hauglund M. J., Tatum F. M., Bayles D. O., Maheswaran S. K., Briggs R. E. Genome Sequences of Mannheimia haemolytica Serotype A1 Strains D153 and D193 from Bovine Pneumonia. 2013. Genome Announc. 1(5). pii: e00848-13.

Harhay G. P., Koren S., Phillippy A. M., McVey D. S., Kuszak J., Clawson M. L., Harhay D. M., Heaton M. P., Chitko-McKown C. G., and Smith T. P. 2013. Complete Closed Genome Sequences of Mannheimia haemolytica Serotypes A1 and A6, Isolated from Cattle.Genome Announc. 1(3). pii: e00188-13.

Hauglund MJ, Tatum FM, Bayles DO, Maheswaran SK, Briggs RE. Genome Sequences of serotype A2 Mannheimia haemolytica isolates D171 and D35 recovered from Bovine Pneumonia. Genome Announc. Accepted for publication February 2, 2015.

Hauglund MJ, Tatum FM, Bayles DO, Maheswaran SK, Briggs RE. Genome Sequences of serotype A6 Mannheimia haemolytica isolates D174 and D38 recovered from Bovine Pneumonia. Genome Announc. Accepted for publication January 29, 2015.

Development of Effective Modified-live Mucosal and Injectable Mannheimia haemolytica Vaccines to Control Respiratory Disease in Ruminant Livestock.

The bacterium Mannheimia haemolytica continues to be a major cause of respiratory disease and economic loss to cattle producers despite the availability of multiple commercial vaccine products and numerous antibiotic countermeasures. Part of the issue is attributable to the relatively short window of opportunity to intervene after calves are first assembled and moved from cow-calf operations to stocker or feeder operations. Vaccine products require a period of time after administration before they are effective, but disease develops quickly in groups of calves stressed by assembly and transportation. Antibiotic treatment affords rapid efficacy, but multi-resistant Mannheimia with associated treatment failure has become a significant problem. ARS scientists at the National Animal Disease Center, Ames, Iowa, developed modified-live strains of several serotypes of Mannheimia haemolytica for use in cattle, sheep, and goats. A goal of this research project is to elicit useful immunity more quickly and to allow alternative routes of delivery to facilitate earlier and more convenient dosage. ARS scientists showed that simple top-dressing of the vaccine strains onto cattle feed elicits relatively high level resistance to lung disease. The data indicate that a single application is likely to be as effective as two doses commonly recommended for conventional vaccines. This route of delivery elicited a detectable mucosal immune response three days after vaccine delivery, more rapid than that

typically associated with injectable vaccine products. This route of vaccine delivery would also enable mass-vaccination of calves prior to shipment without necessitating individual calf handling, further speeding the response compared to conventional vaccination after arrival at destination.

Scientific Publications

Briggs R. E., Tabatabai L. B., and Tatum F. M. 2012. Mucosal and parenteral vaccination against pneumonic pasteurellosis in cattle with a modified-live in-frame lktA deletion mutant of Mannheimia haemolytica. Microb. Pathog. 52(5):302-9.

Briggs R. E., Hauglund M. J., Maheswaran S. K., and Tatum F. M. 2013. Bivalent vaccination against pneumonic pasteurellosis in domestic sheep and goats with modified-live in-frame lktA deletion mutants of Mannheimia haemolytica. Microb. Pathog. 64:43-7.

Evaluation of Vitamin D Levels on the Response to Challenge with Bovine Respiratory Syncytial Virus.

A study conducted by ARS scientists at the National Animal Disease Center, Ames, Iowa, evaluated varying vitamin D levels on the response to bovine respiratory syncytial virus (BRSV) infection. Calves were fed milk replacer diets differing in levels of vitamin D to establish two treatment groups, which were either sufficient or deficient in vitamin D. Animals were then experimentally infected with BRSV. These experiments showed for the first time that BRSV infection turns on the vitamin D pathway in the lung. Importantly, ARS scientists observed that proteins frequently inhibited by vitamin D are, in fact, either significantly increased or not affected in the lungs of BRSV-infected calves with high levels of vitamin D in their blood. The results from these experiments indicated that while vitamin D does have an immunomodulatory role during BRSV infection, there was no significant impact on pathogenesis during the early phases of BRSV infection. Further research is on-going to evaluate the potential role of vitamin D as adjunct therapy to protect calves against respiratory pathogens.

Scientific Publication

Sacco, R.E., B.J. Nonnecke, M.V. Palmer, W.R. Waters, J.D. Lippolis, and T.A. Reinhardt. 2012. Differential expression of cytokines in response to respiratory syncytial virus infection of calves with high or low circulating 25-hydroxyvitamin D3. PLos One 7:e33074.

Rate and Source of Genetic Change in Bovine Viral Diarrhea Virus.

Bovine viral diarrhea virus (BVDV) is a ubiquitous viral pathogen of cattle worldwide. It spreads readily between animals and herds. Additionally, BVDV can infect pregnant cattle that can result in birth of a calf that is persistently infected (PI) and spreads the virus for life. It is well established that there is considerable genetic variability between strains of BVDV and it is not clear how rapidly these changes occur or what drives genetic change. The first study investigating genetic change in BVDV found that a single infection of a pregnant dam resulted in more changes than a virus that caused numerous acute infections over a large geographic area over greater than a year. A single infection that resulted in a PI calf showed from 22 to 48 nucleotide changes in the genome of the virus from the PI calf than that in the infecting PI virus. On the other hand, a virulent BVDV strain passing through dairy herds in Canada and northeast United States showed only a few changes ranging from 6 to 40 nucleotides in a virus isolated 1

year after the initial outbreak. In a second study, rates of genetic changes were looked at in pregnant animals as well as acutely-infected steers (non-pregnant) to determine rate of change. This analysis revealed that the rate of change in acutely infected steers was low, ranging from 4 to 8 nucleotide differences in a single infection. In the pregnant cattle, a higher rate of change was seen, importantly, it was found that the majority of the changes were introduced during the infection of the dam at 6 days post-infection. The PI virus from calf only had a small number of additional nucleotide changes (2-4). Infection of the fetus has been shown to occur at around 14 days post-infection, indicating that most genetic change is introduced in the dam before infection of the fetus. These results showed that infection of a pregnant dam results in 2.5 to 8 fold higher numbers of nucleotide changes than acute infection of non-pregnant animals. This provides greater evidence for the need to prevent PI infections and rapidly eliminate PI calves from herds when detected.

Scientific Publications

Neill JD, Newcomer BW, Marley SD, Ridpath JF, Givens MD. 2011. Genetic change in the open reading frame of bovine viral diarrhea virus is introduced more rapidly during the establishment of a single persistent infection than from multiple acute infections. Virus Res. 158:140-145.

Neill JD, Newcomer BW, Marley SD, Ridpath JF, Givens MD. 2012. Greater numbers of nucleotide substitutions are introduced into the genomic RNA of bovine viral diarrhea virus during acute infections of pregnant cattle than of non-pregnant cattle.Virol. J. 9:150.

Analysis of the Cytopathic Strain of BVDV from 13 Persistently Infected Animals from a Single Herd.

The majority of Bovine viral diarrhea virus (BVDV) strains are of the non-cytopathic biotype, those that do not kill infected cells. When these viruses infect a pregnant animal, the virus will cross the placenta, infect the fetus and cause a persistent infection (PI). This persistent infection is life long and the calf will spread virus wherever it goes, infecting herd mates and spreading the disease. Occasionally, a virus in a PI animal will mutate, either by incorporation of host sequences or viral genomic duplication, resulting in a cytopathic virus. The cytopathic virus does kill infected cells. This will lead to mucosal disease in the PI animal, which is invariably fatal. One herd in South Dakota had 34 identified PIs. After the animals were 1 year old, thirteen animals died of mucosal disease. The cytopathic viruses from these animals were isolated and the genomic changes characterized. It was found that these viruses all contained a cellular insert derived from the messenger RNA transcript, a common finding in BVDV type 2 cytopathic strains. However, the host sequences in all of the viruses were found to be of the same length and had the same nucleotide borders. This indicated that there was only a single mutation event within the herd that gave rise to a cytopathic virus. This cytopathic virus spread to the other animals in the herd, resulting in the fatal disease. This is the first report following such a large number of PI animals and the behavior of their persistent viruses.

Scientific Publication

Darweesh M. F., Rajput M. K., Braun L. J., Ridpath J. F., Neill J. D., Chase C. C. 2015. Characterization of the cytopathic BVDV strains isolated from 13 mucosal disease cases arising in a cattle herd. Virus Res. 195:141-147.

Mycoplasma bovis Isolated from Cattle and Bison have Different Genetic Variants, Potentially Associated with Impact in Health.

Mycoplasma bovis causes mastitis, pneumonia and arthritis in cattle and is the bacterium isolated most frequently from bovine respiratory disease complex (BRDC). Recently, M. bovis has emerged as a significant health problem in bison, causing both respiratory disease and reproductive problems, including abortion. In cattle, M. bovis is typically identified as one of several infectious agents acting in concert to cause disease, but in bison it causes severe disease in the absence of other pathogens. Whether isolates associated with disease in cattle are genetically distinct from bison disease-causing isolates and, if so, whether they pose an added risk to the health of cattle, is unknown. This study describes the development of a molecular typing method for M. bovis, called multilocus sequence typing (MLST), which distinguishes between isolates based on comparison of DNA sequences from several genes important for basic, life-sustaining activities of the bacterium. Additionally, ARS scientists created and made publicly available a curated MLST database for M. bovis to which researchers can contribute data from newly evaluated isolates. Based on the evaluation of 94 cattle isolates from all over the world and 42 bison isolates from the United States and Canada, ARS scientists identified 32 different types of M. bovis. Those found in cattle are different from those found in bison, suggesting recent disease outbreaks in bison may be due to the emergence of new genetic variants and raising concern as to whether those variants may have heightened virulence in cattle. The MLST scheme and database provide novel tools for exploring the population structure of M. bovis and tracking the evolution and spread of strains, including possible spread to cattle of variants currently found only in bison. This study exposes the need to further evaluate newly identified variants so that their potential to negatively impact cattle health and production can be understood.

Scientific Publication

Register, K.B., Thole, L., Rosenbush, R.F. and Minion, F.C. 2015. Multilocus sequence typing of Mycoplasma bovis reveals host-specific genotypes in cattle versus bison. Vet. Microbiol. 175:92-98.

Experimental Evidence Identifying Extended Bighorn Sheep Survival when Co-housed with Domestic Sheep in the Absence of Mycoplasma ovipneumoniae.

Bighorn sheep have experienced population limiting pneumonia, and attention has focused on exposure to domestic sheep as a potential source. Previous studies showed co-housing bighorn sheep with domestic sheep resulted in rapid, lethal pneumonia in bighorn sheep. ARS scientists in Pullman, Washington, worked in collaboration with researchers from Washington State University and the Idaho Department of Fish and Game to test if using domestic sheep free of Mycoplasma ovipneumoniae in co-housing would influence bighorn sheep survival. Three out of four bighorn sheep lived through the test period of more than 100 days, and the fourth died with pneumonia only after 90 days of co-housing. This unprecedented survival was similar to co-housing bighorn sheep with other ungulate species. These results provide prospective, experimental evidence for the importance of Mycoplasma ovipneumoniae in bighorn sheep pneumonia and support development of intervention strategies based on Mycoplasma ovipneumoniae.

Scientific Publication
Besser, T.E., Cassirer, F.E., Yamada, C., Potter, K.A., Herndon, C., Foreyt, W.J., Knowles Jr, D.P., Srikumaran, S. 2012. Survival of bighorn sheep (Ovis canadensis) commingled with domestic sheep (Ovis aries) in the absence of Mycoplasma ovipneumoniae. Journal of Wildlife Diseases. 48(1):168-172.

Antibodies Specific for Ovine Herpesvirus 2 (OvHV-2) Proteins Block Virus Infection and Prevent Malignant Catarrhal Fever in Rabbits.
Identification of OvHV-2 proteins that can stimulate a protective immune response is a prerequisite to the development of a vaccine for sheep-associated Malignant Catarrhal fever. ARS scientists in Pullman, Washington, in collaboration with researchers from Washington State University demonstrated that antibodies against OvHV-2 glycoproteins (gB, gH, and gL) are capable of blocking virus entry into cells using a recently developed rabbit infection model. Antibodies specific for OvHV-2 proteins were produced in rabbits using a biolistic DNA delivery system referred to as a gene gun. The results showed that antibodies against gB, gH, gL completely blocked viral infection and prevented disease development in rabbits, indicating that OvHV-2 gB, gH, and gL are suitable targets for a vaccine aimed at stimulating protective immune responses.

Scientific Publications
Li H, Cunha CW, O'Toole D, Nicola AV, Knowles DP, Taus NS. 2013. Development of an in vivo system to measure antibody-blocking of ovine herpesvirus 2 entry. J. Virol. Meth. 188:104-107.

Cunha CW, Knowles DP, Taus NS, O'Toole D, Nicola AV, Aquilar HC, Li H. 2015. Antibodies to ovine herpesvirus 2 glycoproteins decrease virus infectivity and prevent malignant catarrhal fever in rabbits. Vet. Microbiol. 175:349-355.

Problem Statement 4B: Porcine Respiratory Diseases

Amino Acid Changes in a Viral Protein Determine the Evolution of Swine Influenza A H3N2 Viruses.
Swine influenza A virus is an endemic and economically important pathogen in pigs with the potential to infect other host species including humans. Pigs may also become infected with human influenza A viruses. The viral hemagglutinin (HA) protein binds virus to cells and is the primary target of protective immune responses and the major component in swine influenza A vaccines. However, as a result of genetic mutations known as antigenic drift, vaccine virus strains must be regularly updated to reflect currently circulating strains. Characterizing how different virus strains in pigs are to the seasonal influenza virus strains in humans is also important in assessing the relative risk of interspecies transmission. ARS scientists at the National Animal Disease Center in Ames, Iowa, found that two primary swine influenza virus strains are currently circulating in the U.S. pig population, but with enough diversity between the HA proteins to suggest updates in vaccine strains are needed. ARS scientists identified specific changes in the HA protein that are likely responsible for differences between the two viruses. These changes may be useful in predicting when vaccines need to be updated. The differences between current seasonal influenza H3N2 strains in humans and those endemic in swine is

enough that population immunity is unlikely to prevent the introduction of human viruses into pigs and vice-versa, reinforcing the need to continuously monitor and prepare for influenza A viruses.

Scientific Publication
Lewis, N.S., Anderson, T.K., Kitikoon, P., Skepner, E., Burke, D.F., Vincent, A.L. 2014. Substitutions near the hemagglutinin receptor-binding site determine the antigenic evolution of influenza A H3N2 viruses in U.S. swine. Journal of Virology. 88(9):4752-4763.

Swine influenza DNA Vaccine.
Swine influenza is a highly contagious viral infection in pigs that significantly affects the pork industry due to weight loss and secondary infections. There is also the potential of a significant threat to public health, as occurred in 2009 when the pandemic H1N1 influenza virus strain emerged from reassortment events among avian, swine, and human influenza viruses within pigs. As classic and pandemic H1N1 strains now circulate in swine, an effective vaccine may be the best strategy to protect the pork industry and public health. Current inactivated-virus vaccines available for swine influenza protect only against viral strains closely related to the vaccine strain, and egg-based production of these vaccines is insufficient to respond to large outbreaks. DNA vaccines are a promising alternative because they can potentially induce broad-based protection with more efficient production methods. ARS scientists in Ames, Iowa, working together with scientists at the National Institutes of Health in Bethesda, Maryland, evaluated the potential of monovalent and trivalent DNA vaccine constructs to elicit immunological responses and protect pigs against viral shedding and lung disease after challenge with pandemic H1N1 or classic swine H1N1 influenza virus. Scientists also compared the efficiency of a needle-free vaccine delivery method to that of a conventional needle/syringe injection. The results of these studies demonstrated that DNA vaccination elicits robust serum antibody and cellular responses after three immunizations and confers significant protection against influenza virus challenge. Needle-free delivery elicited improved antibody responses with the same efficiency as conventional injection and could be considered for development as a practical alternative for vaccine administration.

Scientific Publication
Gorres J.P., Lager K.M., Kong W.P., Royals M., Todd J.P., Vincent A.L., Wei C.J., Loving C.L., Zanella E.L., Janke B., Kehrli M.E. Jr, Nabel G.J., Rao S.S. 2011. DNA vaccination elicits protective immune responses against pandemic and classic swine influenza viruses in pigs. Clin Vaccine Immunol. Nov;18(11):1987-95. Epub 2011 Sep 14.

A Genetically-Engineered Swine Influenza Vaccine Confers Cross-Protection against Variant Virus Strains.
It is widely recognized that the diversity of swine influenza virus (SIV) strains impedes the effective immunization of swine herds. This is of great concern as emerging variant swine influenza viruses could emerge into the human population. New variant viruses may also have significant negative economic impact on the swine industry. Therefore, the evaluation of modern vaccine technologies for SIV in the swine host is important for achieving greater control of emerging variant virus strains in swine populations and limiting the risk of transmission to humans. Live virus vaccines are considered more effective than inactivated or non-replicating

virus vaccines as inducers of cellular immunity, but all licensed SIV vaccines in the United States are based on inactivated virus antigens. ARS scientists at the National Animal Disease Center, Ames, Iowa, used molecular approaches to construct mutated H3N2 SIV genomes that result in attenuated replication properties. Truncation of a key viral protein (NS1) used by influenza virus to evade the host immune system produced a mutant virus with restricted replication in the swine respiratory tract but strong immunogenic properties. Intranasal inoculation of pigs with this virus resulted in robust protection against homologous challenge and significantly reduced viral replication and clinical signs upon challenge with a heterologous H1N1 SIV strain.

Scientific Publication
Kappes M.A., Sandbulte M.R., Platt R., Wang C., Lager K.M., Henningson J.N., Lorusso A., Vincent A.L., Loving C.L., Roth J.A., Kehrli M.E. (2011). Vaccination with NS1-truncated H3N2 swine influenza virus primes T cells and confers cross-protection against an H1N1 heterosubtypic challenge in pigs. Vaccine. 2012 Jan 5;30(2):280-8. Epub 2011 Nov 7.

A Panviral Microarray for Detection of Swine Respiratory Viruses in Clinical Samples.
The continuous emergence of swine viruses has elevated the need for diagnostic platforms that have the ability to detect many known, novel, and emerging pathogenic agents simultaneously. Panviral DNA microarrays represent the most robust approach for massively parallel viral surveillance and detection. The Virochip is a panviral DNA microarray that is capable of detecting all known viruses, as well as novel viruses related to known viral families, in a single assay and has been used to successfully identify known and novel viral agents in clinical human specimens. However, the usefulness and the sensitivity of the Virochip platform have not been tested on a set of clinical veterinary specimens with the high degree of genetic variance that is frequently observed with swine virus field isolates. ARS scientists in Ames, Iowa, investigated the utility and sensitivity of the Virochip to positively detect swine viruses in both cell culture-derived samples and clinical swine samples. The Virochip successfully detected porcine reproductive and respiratory syndrome virus (PRRSV) in serum and influenza A virus in lung lavage fluid. The Virochip also successfully detected porcine circovirus type 2 (PCV2) and porcine respiratory coronavirus (PRCV) in turbinate tissue homogenate. Collectively, these data demonstrate that the Virochip can successfully detect pathogenic viruses frequently found in swine in a variety of solid and liquid specimens, such as turbinate tissue homogenate and lung lavage fluid, as well as antemortem samples, such as serum.

Scientific Publication
Nicholson T.L., Kukielka D., Vincent A.L., Brockmeier S.L., Miller L.C., Faaberg K.S. 2011. Utility of a panviral microarray for detection of swine respiratory viruses in clinical samples. J Clin Microbiol. 2011 Apr;49(4):1542-8. Epub 2011 Jan 26.

Swine influenza Vaccine-associated Enhanced Respiratory Disease (VAERD) Characterized in Pigs.
Influenza A virus causes a respiratory disease in pigs similar to that in humans. Inactivated influenza virus vaccine use in swine has increased over the past 10 years in an effort to prevent disease and transmission of the virus. Inactivated vaccines work well when pigs are exposed to influenza viruses that are the same virus used to produce the vaccine; however, vaccine efficacy

is reduced when pigs are infected with different or new strains. ARS scientists at the National Animal Disease Center in Ames, Iowa, found that pigs administered an inactivated swine influenza A vaccine followed by infection with the pandemic human influenza A virus (2009) demonstrated more severe disease compared to non-vaccinated pigs infected with the same virus. Pigs with VAERD demonstrated greater percentages of affected lungs compared to controls, the microscopic damage was more severe with distinct lesions, and had elevated immune factors associated with inflammation and disease in the lungs. Active surveillance and monitoring of the quality of match between vaccine strains and strains infecting swine herds is needed to prevent vaccine mismatch and VAERD in commercial swine. Future vaccines that stimulate improved immune responses for differing influenza viruses will be important to prevent infection and clinical disease in commercial swine production, as well as potential virus transmission to humans.

Scientific Publications

Vincent, A.L., Ma, W., Lager, K.M., Richt, J.A., Janke, B.H., Sandbulte, M.R., Gauger, P.C., Loving, C.L., Webby, R.J., Garcia-Sastre, A., 2012. Live Attenuated Influenza Vaccine Provides Superior Protection from Heterologous Infection in Pigs with Maternal Antibodies without Inducing Vaccine-Associated Enhanced Respiratory Disease. J Virol 86, 10597-10605.

Gauger, P.C., Vincent, A.L., Loving, C.L., Henningson, J.N., Lager, K.M., Janke, B.H., Kehrli, M.E., Jr, Roth, J.A., 2012. Kinetics of lung lesion development and pro-inflammatory cytokine response in pigs with vaccine-associated enhanced respiratory disease induced by challenge with pandemic (2009) A/H1N1 influenza virus. Vet Pathol 49, 900-912.

Gauger, P.C., Vincent, A.L., Loving, C.L., Lager, K.M., Janke, B.H., Kehrli, M.E., Jr., Roth, J.A., 2011. Enhanced pneumonia and disease in pigs vaccinated with an inactivated human-like (delta-cluster) H1N2 vaccine and challenged with pandemic 2009 H1N1 influenza virus. Vaccine 29, 2712-2719.

Vincent, A.L., Lager, K.M., Janke, B.H., Gramer, M.R., Richt, J.A., 2008. Failure of protection and enhanced pneumonia with a US H1N2 swine influenza virus in pigs vaccinated with an inactivated classical swine H1N1 vaccine. Vet Microbiol 126, 310-323.

A Rapid Diagnostic Test for Pseudorabies Surveillance.

Pseudorabies virus (PRV), the cause of Aujeszky's disease, was eradicated from U.S domestic swine herds but continues to circulate in the feral swine population and thus continues to pose a threat for the commercial swine industry. A critical need for the current PRV surveillance program in the United States is the rapid detection of PRV. ARS scientists validated a real-time PCR assay for pseudorabies virus surveillance. Real-time polymerase chain reaction (real-time PCR) is a valuable diagnostic technique that can rapidly identify infectious agents in clinical specimens. Diagnostic performance of the real-time PCR assay developed as a testing method confirmed that it is a rapid, accurate assay that is adaptable to a variety of PCR platforms currently in use by diagnostic laboratories around the world and can provide reliable results on an array of clinical samples.

Scientific Publication
Zanella, E.L., Miller, L.C., Lager, K.M., Bigelow, T.T. 2012. Evaluation of a real-time polymerase chain reaction assay for pseudorabies virus surveillance purposes. Journal of Veterinary Diagnostic Investigation. 24(4):739-745.

Determination of the Genetic Basis by which Haemophilus parasuis Causes Disease and Development of More Efficacious Vaccines.
Haemophilus parasuis is a bacterium that causes Glässer's disease in swine, a disease characterized by chronic debilitation and often death that costs the swine industry millions in losses annually. However, not all strains of the bacterium cause disease. To date, little is known about genetic differences among H. parasuis strains and the genetic factors that contribute to its ability to cause disease. ARS scientists at the National Animal Disease Center in Ames, Iowa, identified 10 strains of H. parasuis with varying ability to cause disease and then determined the DNA genomic sequence of these strains. Comparative genomic analysis of the different strains has identified several significant differences that likely contribute to the variability in disease caused by these strains. Subsequent studies have demonstrated that strains that don't cause disease can be used as vaccines against strains that do and are leading to improved vaccines against H. parasuis for pigs.

Scientific Publications
Brockmeier SL, Loving CL, Mullins MA, Register KB, Nicholson TL, Wiseman BS, Baker RB, Kehrli ME, Jr. 2013. Virulence, transmission, and heterologous protection among four isolates of Haemophilus parasuis. Clin Vaccine Immunol. 20:1466-1472.

Kuehn JS, Register KB, Phillips GJ. 2013. Draft genome sequences for 10 isolates of the swine pathogen Haemophilus parasuis. Genome Announc. 1:5.

Brockmeier, S.L., Register, K.B., Kuehn, J.S., Nicholson, T.L., Loving, C.L., Bayles, D.O., Shore, S.M, Phillips, G.J. 2014. Virulence and draft genome sequence overview of multiple strains of the swine pathogen Haemophilus parasuis. PLOS One: 9(8) e103787. DOI: 10:1371/journal.pone.0103787.

Porcine Granulocyte-colony Stimulating Factor (G-CSF) Delivered via Replication-defective Adenovirus Induces a Sustained Increase in Circulating Peripheral Blood Neutrophils.
The use of cytokines that stimulate the immune system as alternatives to antibiotics is a promising area for biotherapeutic use to prevent and combat infectious disease. ARS scientists at the National Animal Disease Center, Ames, Iowa, have investigated the potential value of using the granulocyte-colony stimulating factor (G-CSF) as a potential alternatives to antibiotics in food-animal production as a possible candidate for pathogenic bacteria in which neutrophils (white blood cells that are the first line of defense against bacterial infections) can provide protection. G-CSF enhances the production and release of neutrophils from bone marrow and is already licensed for use in humans. A limitation of cytokines is their short half-life, which may limit their usefulness as a one-time injectable in production-animal medicine. ARS scientists found that the administration of recombinant G-CSF induced a transient increase in neutrophils (neutrophilia) in pigs; however, delivery of porcine G-CSF inserted in a replication-defective adenovirus (Ad5) vector significantly increased the neutrophilia pharmacodynamics effect. Pigs

given one injection of the Ad5-G-CSF had a neutrophilia that peaked between days 3-11 post-treatment and neutrophil counts remained elevated for more than 2 weeks. Neutrophils from Ad5-G-CSF treated pigs were fully functional based on laboratory tests, demonstrating that G-CSF may be an effective alternative to antibiotics for treating bacterial pathogens that are susceptible to neutrophils.

Scientific Publication
Loving C.L., Kehrli M.E., Brockmeier S.L., Bayles D.O., Michael D.D., Schlink S.N., Lager K.L. 2013. Porcine granulocyte-colony stimulating factor (G-CSF) delivered via replication-defective adenovirus induces a sustained increase in circulating peripheral blood neutrophils. Biologicals 41(6): 368-376.

Protection and Improved Immune Response to Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) with Type I Interferon.
PRRSV is the most costly viral disease for pig producers. Interferon-alpha (IFN-alpha) is an antiviral agent produced by cells in the body as a first line of defense against viral infection as well as for activation of the adaptive immune response. Little interferon-alpha is produced during PRRSV infection in pigs, which is potentially the reason for the delay in an adaptive immune response to the virus. ARS scientists at the National Animal Disease Center in Ames, Iowa, found that giving IFN-alpha to pigs resulted in decreased replication of PRRSV, less severe disease, and an improved adaptive cell mediated immune response. These results indicate that IFNα could be used therapeutically and improve the immune response induced by vaccines, which currently do not protect well against PRRSV infection.

Scientific Publication
Brockmeier SL, Loving CL, Nelson EA, Miller LC, Nicholson TL, Register KB, Grubman MJ, Brough DE, Kehrli ME. 2012. The presence of alpha interferon at the time of infection alters the innate and adaptive immune response to porcine reproductive and respiratory syndrome virus. Clin Vaccine Immunol. 19:508-514.

A Secretion System Plays a Role in the Persistence, Disease and Immunosuppression Caused by Bordetella bronchiseptica.
The porcine respiratory disease complex (PRDC) is a multifactorial disease complex of swine caused by numerous viruses, bacteria and adverse environmental conditions. In its various forms PRDC is the most costly problem to the swine producer today. There is a need for a better understanding of the pathogens that contribute to the PRDC and the specific interactions between these pathogens and the host. *Bordetella bronchiseptica* is a bacterium that is widespread in swine herds and plays multiple roles in respiratory disease, causing both upper-respiratory illness and pneumonia, and predisposes pigs to infection with other bacteria. The type III secretion system (T3SS) is a needle-like structure that the bacterium uses to inject proteins directly into host cells to aid in the infection process. ARS scientists at the National Animal Disease Center in Ames, Iowa, constructed a mutant of B. bronchiseptica that does not make the T3SS to determine what role this system plays in colonizing the respiratory tract, causing disease, and transmitting pig-to-pig. Infection with the Bordetella T3SS mutant was cleared more rapidly, indicating the T3SS is required for the bacteria to persist in the pig's respiratory tract. Additionally, disease was milder in pigs infected with the Bordetella T3SS mutant. The presence

of the T3SS was found to hinder the pig's immune response to Bordetella, which likely plays a role in the inability of swine to clear the bacteria and is why Bordetella can normally persist in the swine respiratory tract for long periods of time. However, the T3SS mutant was still capable of transmitting from one pig to another, demonstrating that even with minimal disease and lower colonization transmission can occur. Targeting proteins secreted by the T3SS for future vaccines will decrease disease caused by Bordetella and potentially reduce secondary bacterial infections as a result of infection with this bacteria.

Scientific Publication
Nicholson TL, Brockmeier SL, Loving CL, Register KB, Kehrli ME Jr., Shore SM. 2013. The Bordetella bronchiseptica type III secretion system is required for persistence and disease severity but not transmission in swine. Infect Immun. 82:1092-1103.

Problem Statement 4C: Poultry Respiratory Diseases

Improving Infectious laryngotracheitis Vaccines.
Infectious laryngotracheitis (ILT) is a highly contagious acute respiratory disease that is continuing to have significant negative impact on poultry production in the United States. One of the key issues concerns current commercial ILT vaccines, which lack the desired safety and/or efficacy profile under field conditions. For protection, chickens are vaccinated multiple times with live strains that were attenuated by either multiple passages in embryonated eggs or in tissue culture. Although these vaccines protect against clinical disease, they have residual virulence that is exacerbated by continued infections of naive birds from productively infected animals and latent carriers. Moreover, the vaccine strains produced in embryonated eggs have been shown to mutate and become more virulent simply by bird-to-bird passage. Because of this characteristic, it is believed that U.S. vaccine strains have mutated to become more virulent and these "revertants" have become the dominant field strains in the poultry population. As a result of this increased virulence in the circulating virus and the use of high-density poultry housing, there is a continuous reservoir of viruses (both virulent and vaccinal) in flocks that is capable of evolving to higher levels of virulence. There is therefore a pressing need to develop safer and more effective ILT vaccines. ARS scientist at the Southeast Poultry Research Laboratory, Athens, Georgia, generated a new generation vaccine platform based on using the Newcastle disease virus (NDV) LaSota vaccine strain to express glycoproteins B (gB) or D (gD) of ILTV using reverse genetics technology. These recombinant NDV-vectored ILT vaccine viruses, rLS/ILTV-gB and rLS/ILTV-gD, were shown to be attenuated and safe for use in poultry, and yet retained growth dynamics, stability, and virus titers during vaccine production that were similar to those of the parental LaSota virus. Importantly, these next generation bivalent vaccines provide protection against both NDV and ILT. This was demonstrated in control efficacy studies where vaccination of specific-pathogen-free chickens conferred significant protection against virulent ILTV and velogenic NDV challenges. Immunization of commercial broilers with rLS/ILTV-gB provided complete protection against clinical disease in more than 90% of challenged birds (less than 10% of the birds showed very mild, transient clinical disease) and a significant decrease of challenge virus shedding (37-fold reduction in ILTV load ($P<0.01$) in tears collected at 5 days post challenge compared to non-vaccinated challenged group); birds immunized with rLS/ILTV-gD developed complete (100%) protection against clinical disease following virulent ILTV challenge with a 22-fold reduction of challenge virus in tear samples

collected at 5 days post challenge compared to non-vaccinated and challenged birds. This level of protection against clinical disease and virus shedding appeared to be similar to that provided by the live attenuated commercial vaccines. In addition, there was no significant impact on body weight gains (that is frequently seen in birds with clinical ILT disease) of birds vaccinated with rLS/ILTV-gB or rLS/ILTV-gD and challenged with virulent ILTV. The results from studies to date suggest that the rLS/ILTV-gB and -gD vaccine viruses are safe, stable, and effective bivalent vaccines that can be mass administered via aerosol or drinking water to large chicken populations. ARS is currently in the process of transferring this vaccine technology to a commercial partner for development, manufacture, and registration.

Scientific Publication
Zhao, W., Spatz, S., Zhang, Z., Wen, G., Garcia, M., Zsak, L., Yu, Q. Newcastle Disease Virus (NDV) Recombinants Expressing Infectious Laryngotracheitis Virus (ILTV) Glycoproteins gB and gD Protect Chickens against ILTV and NDV Challenges. J. Virol. 88:8397-8406. 2014.

Improved Vaccination Protocols to Control Avian Mycoplasmosis.
Avian mycoplasmosis, primarily caused by Mycoplasma gallisepticum (MG), remains a significant problem to the poultry industry. Consequences of MG infections are significant and may include mortality, carcass condemnation, and reduced egg production, hatchability, feed efficiency, weight gain, and associated medication costs. Due to the current lack of an effective means of control, intense biosecurity and biosurveillance remain the primary line of defense against MG infections. However, MG is considered to be endemic in commercial egg facilities and is commonly associated with backyard poultry flocks and outbreaks among commercial meat-type poultry necessitating further means of control. Antibiotics have been demonstrated ineffective at clearing MG infections and with the current trend toward reduced reliance on these substances, live attenuated vaccines (LAVs) and bacterin-based vaccines are currently widely-used to minimize the losses associated with wild-type MG outbreaks. As these vaccines represent a primary means of protection against MG-associated losses, associated vaccination strategies and protocols should be optimized to maximize protection afforded to the vaccinated host. Toward increasing the efficiency of vaccination protocols, ARS scientists demonstrated positive outcomes when administering multiple MG LAVs to maximize protection.

Scientific Publications
Purswell, J.L., Evans, J.D., and Branton, S.L. 2011. Serologic response of roosters to gradient dosage levels of a commercially available live F strain-derived Mycoplasma gallisepticum vaccine over time. Avian Dis. 55(3):490-494.

Evans, J.D., Leigh, S.A., Purswell, J.L., Collier, S.D., Kim, E.J., Boykin, D.L., and Branton, S.L. 2015. The impact of deposition site on vaccination efficiency of a bacterial-based poultry vaccine. Poult Sci. Submitted 1/27/15.

Jacob, R., Branton, S.L., Evans, J.D., Leigh, S.A., and Peebles, E.D. 2014. Effects of live and killed Mycoplasma gallisepticum vaccinations on the performance characteristics of commercial layer chickens. Poult Sci. 93:1403-1409.

Jacob, R., Branton, S.L., Evans, J.D., Leigh, S.A., and Peebles, E.D. 2015. Effects of different

vaccine combinations against Mycoplasma gallisepticum on the internal egg and eggshell characteristics of commercial layer chickens. Poult Sci. Accepted 1/5/15.

Comparative Genome Analysis of Avian Pasteurella multocida Reveals Candidate Genes Involved in Fitness and Pathogenicity.

The bacterium Pasteurella multocida is the cause of a number of diseases in domestic ruminants, swine, and poultry. In both domestic and wild avian species it causes a highly contagious and severe septicemic disease known as fowl cholera. To serve as a resource for future research, and to identify potential targets for vaccine or therapeutic intervention, ARS scientists at the National Animal Disease Center, Ames, Iowa, obtained the genome sequence of strains X-73 and P1059, P. multocida, highly-virulent strains originally recovered from chicken and turkey hosts respectively. These were compared to the reference strain Pm70, which was isolated from a chicken but which is virtually avirulent in avian hosts. Analysis revealed 336 unique genes in P1059 and X-73, which are absent in strain Pm70. Of particular interest is the identification of novel sugar transport systems including an operon that allows the utilization and transport of L-fucose. Such systems have been identified in enteric bacteria such as Camplyobacter and E. coli, which may be an adaptation to the gut environment relatively rich in fucose-containing mucin. Substantial variation between Pm70 and the virulent avian isolates was observed in a number of core outer membrane proteins, including a filamentous hemagglutinin gene previously demonstrated to be important in avian disease. The identification of virulence genes contributes to the rational design of vaccines that are efficacious against these important poultry respiratory pathogens.

Scientific Publications

Johnson T. J., Abrahante J. E., Hunter S. S., Hauglund M., Tatum F. M., Maheswaran S. K., and Briggs R. E. 2013. Comparative genome analysis of an avirulent and two virulent strains of avian Pasteurella multocida reveals candidate genes involved in fitness and pathogenicity. BMC Microbiol. 13:106.

Abrahante J. E., Johnson T. J., Hunter S. S., Maheswaran S. K., Hauglund M. J., Bayles D. O., Tatum F. M., and Briggs R. E. 2013. Draft Genome Sequences of Two Virulent Serotypes of Avian Pasteurella multocida. Genome Announc. 2013 Jan;1(1). pii: e00058-12.

Tatum F. M., Tabatabai L. B., Briggs R. E. 2012. Cross-protection against fowl cholera disease with the use of recombinant Pasteurella multocida FHAB2 peptides vaccine. Avian Dis. 56(3):589-91.

Genome Sequences of Ornithobacterium rhinotrachealis to Support Development of Novel Countermeasures Against Poultry Respiratory Disease.

Ornithobacterium rhinotracheale is a global pathogen of significant economic importance, causing pneumonia and airsacculitis in farmed turkeys and chickens as well as a variety of other domesticated and wild birds. The bacterial products critical for causing disease have not been identified. Attempts to control disease by the use of vaccines have met with limited success, largely due to the inability of any single strain to protect against the variety of strains that circulate in avian species. Scientists at the National Animal Disease Center, Ames, Iowa, have sequenced the genomes of two genetically distinct, disease-causing isolates and analyzed and

compared the number and variety of products they encode. These data provide a novel opportunity to understand, at the genetic level, how O. rhinotracheale causes disease and to identify potentially cross-protective proteins, thereby facilitating the development of efficacious vaccines that will reduce the economic impact of the bacterium on poultry production.

Scientific Publications

Zehr, E.S., Bayles, D.O., Boatwright, W.D., Tabatabai, L.B. and Register, K.B. 2014. Complete genome sequence of Ornithobacterium rhinotracheale strain ORT-UMN 88. Stand. Genomic Sci. 9:16.

Zehr, E.S., Bayles, D.O., Boatwright, W.D., Tabatabai, L.B. and Register, K.B. 2014. Genome sequence of Ornithobacterium rhinotracheale strain H06-030791. Stand. Genomic Sci. 9:14.

A Practical Means to Administer Bacteriophage in Commercial Poultry.

It has been difficult to develop a practical means to administer bacteriophage in commercial poultry facilities. ARS scientists in Fayetteville, Arkansas, have developed two new models for colibacillosis, either litter application of Escherichia coli or the use of seeder birds challenged with E. coli, combined with cold stress. These models have enabled them to demonstrate the efficacy of environmental augmentation with bacteriophage to prevent non-clinical colibacillosis. This provides an effective, natural, and safe alternative to antibiotics to prevent and treat an important respiratory disease in commercial poultry production facilities and can be extended to other animal production systems.

Scientific Publications

El-Gohary, F.A., Huff, W.E., Huff, G.R., Rath, N.C., Zhou, Z.Y., Donoghue, A.M. 2014. Environmental augmentation with bacteriophage prevents colibacillosis in broiler chickens. Poult. Sci. 93:2788-2792.

Huff, W.E., Huff, G.R., Rath, N.C., Donoghue, A.M. 2013. Method of administration affects the ability of bacteriophage to prevent colibacillosis in 1-day-old broiler chickens. Poult. Sci. 92:930-934.

Yeast Extract Feed Supplementation Prevents Clostridial Dermatitis.

Clostridial dermatitis (CD) is a costly production disease of commercial turkeys. ARS scientists at Fayetteville, Arkansas, have developed an experimental model for this disease using injection with a stress hormone that suggests this disease is related to production stressors and has a respiratory component. Using this model, they have demonstrated that yeast extract feed supplementation can decrease the incidence of CD. This research provides a low cost alternative to antibiotics for preventing this disease in turkey production.

Scientific Publication

Huff, G.R., Huff, W.E., Rath, N.C. 2014. Effects of vitamin D and yeast extract supplementation on turkey mortality and clostridial dermatitis incidence in a dexamethasone immunosuppression model. Avian Dis. 58:572-578.

Component 5: Enteric Diseases

Rationale for the research:
Enteric diseases affect animals and humans universally and are the cause of significant production losses and mortality. A number of enteric pathogens are zoonotic and considered food safety pathogens that pose major public health concerns. The problems associated with food safety pathogens are addressed under National Program 108, Food Safety. Endemic enteric diseases of livestock and poultry remain economically important causes of production losses. Although many enteric diseases can be prevented through sound biosecurity measures and good management practices, significant scientific gaps remain in our understanding of commensal (harmless beneficial microorganisms) versus pathogenic infections, polymicrobial infections and enteric disease complexes, disease transmission, and the ecological and host interactions that lead to disease and production losses. With the continued concern over the use of antibiotics in animal production, there is a need to find safe and practical alternatives to prevent and control enteric diseases. Research is needed to identify the pathogens responsible for many enteric diseases, molecular tools for epidemiological studies, and the discovery of improved diagnostics and vaccines that can be integrated in the design of effective prevention and control programs.

The ARS animal health enteric diseases research program focused on a key priority enteric disease of cattle, Johne's disease; and more broadly on enteric diseases of poultry with an emphasis on the gut microbiome and its impact on nutrition and health.

Stakeholders who completed the 2011 retrospective electronic survey representing the dairy industry ranked Johne's disease as their 1st priority; stakeholders representing the turkey industry ranked Poult Enteric Mortality Syndrome (PEMS) as the 3rd most important disease after avian influenza and avian pneumovirus; and stakeholders representing the poultry broiler industry ranked enteric diseases as their 4th priority with Runting-Stunting Syndrome of broilers (RSS) the most important disease.

Research Needs:
Completion of the sequencing of the Mycobacterium paratuberculosis genome provides new research tools to identify M. paratuberculosis-specific genes and proteins that may be useful as diagnostic tools or vaccine candidates. Genomic and proteomic analyses of M. paratuberculosis are needed to identify immunogens that may be differentially expressed in subclinical and clinical stages of disease. In concert with studies in microbial genomics, studies on host immune responses during the different stages of disease are needed to ascertain potential mechanisms that contribute to inflammation and disease and provide potential mitigation strategies. Unique microbial genomic sequences and host responses are needed to implement a technology-driven vaccine discovery program.

Poultry enteric diseases include Poult Enteritis Mortality Syndrome (PEMS), Runting-Stunting Syndrome of broilers (RSS), as well as several unclassified enteric diseases. The exact causes of many of these diseases remain unknown. PEMS affects young turkeys and is probably the most severe form of enteric disease in that species. PEMS was first reported in North Carolina in 1991. Since then, PEMS and similar disease conditions have been reported in most regions where turkeys are commercially produced including; the Southeastern United States, Texas,

California, Arkansas, and Missouri. Since its emergence in 1991, outbreaks of PEMS have cost the turkey industry millions of dollars in losses annually. Although there are no detailed financial studies, it is estimated that PEMS outbreaks cost the North Carolina turkey industry $34 million in losses in 1995 alone, making it the most economically devastating disease of turkeys to date. The exact cause of PEMS is unknown but is thought to be associated with avian astrovirus, avian reovirus, and avian rotavirus infections. Determining the cause of PEMS and other enteric diseases of poultry has been difficult because many enteric viruses cannot be grown in the laboratory and available virus detection tests lack sensitivity and specificity. Moreover, enteric diseases can be caused by two or more infectious agents, working independently but causing clinically similar conditions, or working in concert in so called polymicrobial infections to form enteric disease complexes with primary and secondary pathogens. Accordingly, molecular tools are needed to identify enteric disease viruses and understand the epidemiology and pathogenic pathways responsible for disease and production losses. There is also a need to understand the processes that regulate host response to enteric infection to develop effective strategies to prevent enteric disease.

The Animal Health Action Plan identified a significant list of research priorities and anticipated products expected from research on enteric diseases and that now serve to help measure the national program's progress during the last 5 years in meeting the needs of cattle and poultry producers. The following list of anticipated products from the Action Plan is followed by the expected impact of the research and a sampling of relevant accomplishments.

Anticipated Products In Action Plan:

- Comparative analyses of the M. paratuberculosis proteome leading to the development of highly sensitive and specific diagnostic tests for detection of M. paratuberculosis for cattle and sheep through identification and characterization of unique bacterial genes and proteins.
- Host immune response analyses to understand the mechanisms of control in early stages of disease and the switch in immunity that results in progression from subclinical to clinical disease.
- An effective vaccine platform that prevents subclinical disease, shedding of M. paratuberculosis, and progression to clinical disease.
- Identify factors associated with the risk of enteric diseases of cattle and poultry.
- Identify modulators of stress in production systems that affect enteric disease development in cattle and poultry.
- Discovery of cytokines and their expression profiles that govern processes involved in host defense during enteric infections in cattle and poultry.
- Identify and characterize pathogens responsible for poultry enteric disease complexes
- Pathogen-specific markers useful for molecular or immunological detection.
- Molecular tools to study the epidemiology and ecology of enteric pathogens.
- Strategies that enhance the clearance of enteric pathogens.
- Discovery of immunointervention strategies to prevent the development of enteric infections.

Impact:
The research program provided information on key host-pathogen responses during the infection process that contribute to the development and application of genomic-based diagnostic tests and vaccines to prevent and control Johne's disease. The poultry enteric research program provided information on the detection of pathogens responsible for enteric disease complexes, understanding the relationship of enteric pathogens to each other and host co-evolution, and tools that enable the prevention and control of enteric diseases.

COMPONENT 5: SELECTED ACCOMPLISHMENTS

Problem Statement 5A: Johne's Disease

Genome Sequencing of Ovine Isolates of Mycobacterium avium subspecies paratuberculosis Provide Insight into Host Association.
Paratuberculosis (Johne's Disease) is a chronic progressive enteric disease characterized clinically by chronic or intermittent diarrhea, emaciation and death. Johne's has worldwide distribution and economic impact on ruminant livestock production. The host responses to Mycobacterium avium subspecies paratuberculosis (MAP) are complex, so understanding the host-pathogen interactions will allow the development of new diagnostic tools and intervention strategies. Recent research by ARS scientists at the National Animal Disease Center, Ames, Iowa, found that goats and sheep are susceptible to MAP infection. Results of genome sequencing showed significant differences in the genome of cattle and sheep isolates of MAP. Using next-generation sequencing technology combined with optimal mapping, additional novel regions of difference between cattle and sheep MAP were determined. Tracking these differences allows understanding of differences in pathogenesis of Johne's between the two host species. This work will facilitate development of improved diagnostic assays more aligned with specific ruminant species that could have worldwide relevance in identifying infected animals and preventing production losses.

Scientific Publication
Bannatine, J.P., Wu, C., Hsu, C. Zhou, S., et al. 2012. Genome sequencing of ovine isolates of Mycobacterium avium subspecies paratuberculosis offers insights into host association. Biomed Central (BMC) Genomics. 13:89. Available: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3337245/pdf/1471-2164-13-89.pdf.

Discovery of MAP Proteins as Potential Vaccine Candidates.
Pools of recombinant Mycobacterium avium subsp. paratuberculosis (MAP) proteins, consisting of an overlapping array of 3 proteins in 4 pools, were used to vaccinate mice. Three of the four protein pools showed some level of protection as determined by lower levels of bacteria found in the tissues of infected mice compared to mice not vaccinated. In addition, immune responses in vaccinated mice showed a shift that would correlate with greater protection. One protein array selected for further study demonstrated reduced tissue colonization and fecal shedding in vaccinated calves. A provisional patent has been granted for use of these recombinant proteins as a vaccine for paratuberculosis. The lack of vaccines for paratuberculosis, particularly vaccines that can discriminate between paratuberculosis and M. bovis infections in the field, makes this a compelling avenue of research. Vaccines could be used to successfully reduce the

spread of infection and level of disease within a herd.

Scientific Publication
Stabel, J.R., Barnhill, A., Bannantine, J.P., Chang, Y.F., Osman, M.A. 2012. Evaluation of protection in a mouse model after vaccination with Mycobacterium avium subsp. paratuberculosis protein cocktails. Vaccine 17;31(1):127-34.

Differences in Shedding of MAP into Milk and Colostrum of Clinically Versus Subclinically Infected Cows
Shedding of Mycobacterium avium subsp. paratuberculosis (MAP) into the milk of infected dams is one mode of transmission to young calves. However, there is very little data to define how much shedding occurs and when it occurs during the lactation period. Upon collection of milk during the 305-day lactation cycles from noninfected cows and cows in subclinical and clinical stages of disease, shedding of the bacteria into colostrum and milk was ascertained by culture and PCR. Results demonstrated that cows in the clinical stage of disease shed MAP more frequently and at higher levels than subclinically infected cows. In addition, the majority of positive milk samples were detected in the early stage of lactation, most closely associated with days 0-14 in milk. This study demonstrates that shedding of MAP into milk is affected by infection status of the cow as well as stage of lactation, providing useful information to producers to help break the cycle of infection within a herd.

Scientific Publication
Stabel, J.R., Bradner, L., Robbe-Austerman, S., Beitz, D.C. 2014. Clinical disease and stage of lactation influence shedding of Mycobacterium avium subspecies paratuberculosis into milk and colostrum of naturally infected dairy cows. J. Dairy Sci. 97(10):6296-6304.

Problem Statement 5B: Enteric Diseases of Poultry

Comparative Metagenomic Analysis of the Intestinal RNA Virome and Bacteriome from Sentinel Birds Placed on Selected Broiler Chicken Farms.
The complex viral and bacterial communities present in the poultry gut influences gut development, immune status, and animal health, each of which can be an indicator of overall performance. Scientists at the Southeast Poultry Research Laboratory, Athens, Georgia, investigated the colonization of specific pathogen free (SPF) chickens by enteric microorganisms under field conditions, and compared the pre-contact intestinal microbiome with the altered microbiome following the contact period. Analysis of the intestinal virome (all viruses present in the gut) from contact birds ("sentinels") placed on farms revealed colonization by members of the Picornaviridae, Picobirnaviridae, Reoviridae, and Astroviridae that were not present in pre-contact birds or present in proportionally lower numbers. Analysis of the sentinel gut bacteriome (all bacteria present in the gut) revealed an altered community in the post-contact birds, notably by members of the Lachnospiracea/Clostridium and Lactobacillus families and genera. Members of the avian enteric Reoviridae and Astroviridae have been well-characterized and have historically been implicated in poultry enteric disease; members of the Picobirnaviridae and Picornaviridae have only relatively recently been described in the poultry and avian gut, and their roles in the recognized disease syndromes and in poultry performance in general have not yet been determined.

Scientific Publication
Day, J.M., Oakley, B.B., Seal, B.S., and Zsak, L. 2015. Comparative analysis of the intestinal bacterial and RNA viral communities from sentinel birds placed on selected broiler chicken farms. PLoS One, in press.

Molecular and Phylogenetic Analysis of a Novel Turkey-origin Picobirnavirus (PBV) and Design of a New Molecular Diagnostic Assay for PBV.
A previous metagenomic analysis of the turkey gut RNA virus community identified novel enteric viruses that may play roles in poultry enteric diseases or in performance problems in general. A novel turkey-origin picobirnavirus (PBV) was initially identified by Southeast Poultry Research Laboratory, Athens, Georgia, scientists in a pooled intestinal sample from turkey poults in North Carolina. Little detailed molecular information exists regarding the family Picobirnaviridae, particularly for the PBVs that have been described in avian species. A new reverse transcriptase–PCR (RT-PCR) diagnostic assay was developed targeting the turkey PBV RNA-dependent RNA polymerase (RdRp) gene, producing an 1135-bp amplicon. This assay was validated using in vitro transcribed RNA and was tested using archived enteric samples maintained at the laboratory and collected from turkey flocks in the southeastern United States. Further, a phylogenetic analysis suggests the turkey PBV is unique because it does not group closely with the recognized PBV genogroups circulating in mammalian hosts.

Scientific Publication
Day, J.M. and Zsak, L. 2014. Molecular and phylogenetic analysis of a novel turkey-origin picobirnavirus. Avian Diseases, 58(1):137-142.

Investigating Moderate to Severe Enteritis Associated with Turkey Enteric Coronavirus.
Circulating in the Southeastern United States and Arkansas during 2012 and 2013.
In cooperation with industry stakeholders, periodic monitoring of poultry flocks in the United States via molecular diagnostic methods has revealed a number of potential enteric viral pathogens in continuous circulation in turkeys and chickens. Recently, turkey integrators in the Southeastern United States and Arkansas experienced an outbreak of moderate to severe enteritis associated with turkey enteric coronavirus (TCoV), and numerous enteric samples collected from turkey flocks in these areas tested positive for TCoV via realtime reverse-transcriptase PCR (RRT-PCR) and next-generation sequencing (Ion Torrent platform). Subsequent sequence and phylogenetic analysis of the TCoV spike glycoprotein and the comparison of outbreak-associated isolates to sequences in the public database were performed. TCoVs investigated during the present outbreak grouped geographically based upon state of origin, and the RRT-PCR assay was a good indicator of subsequent seroconversion by TCoV-positive turkey flocks. Further, the TCoV outbreak did not appear to be associated with a concomitant outbreak of infectious bronchitis in broiler flocks in the same region.

Scientific Publication
Day, J.M., Gonder, E., Jennings, S., Rives, D., Robbins, K., Tilley, B. and Wooming, B. 2014. Investigating turkey enteric coronavirus circulating in the Southeastern United States and Arkansas during 2012 and 2013. Avian Diseases, 58(2):313-317.

Component 6: Parasitic Diseases

Rationale for the research:
Parasites represent one of the most diverse groups of organisms that live on a host (ectoparasites) or within a host (endoparasites) and are responsible for hundreds of insidious diseases ranging from enteric diseases to vector-borne hemoparasitic infections. The livestock and poultry industries are severely affected by significant losses in animal production due to lower weight gain, anemia, diarrhea, and death. Nematode infections in cattle and the cost of combating these parasites costs beef producers over $1 billion per year. Many parasites are invasive and exotic to the United States and impact international trade. With the advent of climate change, parasites may spread to new areas increasing their impact on various animal species. Most importantly, the emergence of drug resistant parasites against many commonly used pharmaceutical drugs has huge economic implications.

Stakeholders who completed the 2011 retrospective electronic survey representing the beef industry ranked anti-parasitic drug resistance as their 5th priority; the dairy industry ranked hemoparasites as their 5th priority; the equine industry ranked diseases that impact international movement of horses such as Piroplasmosis (Equine Babesiosis) as their 1st priority; and the sheep and goat industries ranked internal parasites as their 2nd priority. The poultry broiler industry identified enteric diseases such as coccidiosis their 4th priority. The National Cattleman's Beef Association (NCBA) Fiscal Year 2005 Emerging Cattle Health and Issues Working Group identified Anaplasmosis on their list of animal disease research priorities.

Research Needs:
Anti-helminthic resistance to drugs such as Ivermectin and Fenbendazole has been observed world-wide in nematodes of cattle and small ruminants, particularly in sheep-producing countries. Although the number of reports is low, there has been documentation of a significant increase in drug resistance in nematodes of cattle. The drug resistance appears to vary according to host, parasite, and region. This is an area of critical importance and research towards identifying and working on developing an understanding of the mechanisms of resistance as well as alternative strategies is critical for successful animal production.

Avian coccidiosis is a serious protozoan disease of poultry. Currently it is the cause of the largest level of antibiotic use. Due to the concerns for the development of antibiotic resistance as a result of the wide scale use of antibiotics, there is a need for the development of alternative strategies for control of coccidia in poultry. As a result, developing an improved vaccine that is cross protective against a number of protozoan species is needed.

Research is needed to discover vector related contributions to the risk of anaplasmosis in areas within the United States characterized as endemic and the discovery of parasite antigen structure associated with high transmission efficiency and control strategies. In addition, developing improved diagnostic and control strategies for equine piroplasmosis is important research due to the fact it is considered a foreign animal disease in the United States. The constant movement of horses internationally for breeding or competition purposes makes the ability to detect the parasite as well as treat infected horses critical to the well-being of the billion dollar equine industry.

Anticipated Products In Action Plan:

- Develop molecular-based techniques to rapidly speciate and quantify Eimeria oocysts in litter samples.
- Develop rapid tests to identify drug resistance markers in Eimeria field isolates.
- Discover recombinant vaccines that are safe and effective against heterologous field challenges with mass vaccination capability to prevent outbreaks of coccidiosis in poultry farms.
- Investigate and document drug resistance related to parasite species; e.g., Haemonchus contortus, H. placei, Cooperia punctata, C. oncophora, Ostertagia ostergii, Nematodirus helvetianus, and trichostrongyles.
- Determine the effect of different production and management systems on the manifestation of drug resistance in sheep, dairy, cow-calf, and feedlot operations.
- Identify molecular probes to better define parasite species in the field.
- Identify molecular markers of drug resistance based on mode of action and measure the allele frequency of parasite genes involved in drug resistance.
- Identify patterns of gene flow in nematode populations to manage drug resistance in different production systems to reduce the impact of drug resistance on productivity.
- Determine the transmission competence of vectors within the United States and trading partners (Canada).
- Develop vaccines which prevent production losses from clinical disease and transmission (transfection technology is the center of our vaccine strategy for babesiosis).
- Determine if current chemotherapeutics for Anaplama marginale and Babesia caballi are effective in clearing persistent infections.

Impact:
A greater understanding of the extent and type of drug resistance in nematodes of U.S. cattle and sheep, especially as related to the type and phase of farm management is critical to the well-being of livestock production systems. Improved molecular probes for speciating nematodes in the farm environment and for identifying markers of drug resistance are needed as the range of species of parasites spread due to climate change. Reduction in the incidence and effects of nematode infections in cattle and sheep by allowing fact-based application of appropriate anti-helminthic compounds is needed as resistance levels to the current products increases. Data supporting and aiding decisions on import/export restrictions and novel vaccines which prevent clinical disease and block vector-borne transmission and the hemoparasites such as Anaplasma or Babesia is critically needed by the various livestock industries.

Avian coccidiosis is an important problem to the poultry industry. Treatment for coccidiosis by antibiotics is of concern for development of antimicrobial resistance as coccidiostat-resistant protozoa are developing. As a result, alternative strategies are critical. Scientists in Beltsville, Maryland, are working to develop alternatives to antibiotics including development of novel vaccines. The research developed will help control this serious problem while reducing the levels of antimicrobial agents administered to poultry.

COMPONENT 6: SELECTED ACCOMPLISHMENTS

Problem Statement 6A: Gastrointestinal (GI) Parasitic Diseases

Determining the Protective Mechanisms for Resistance and Protection Immunity to Helminth Infection in Ruminants.
Parasites are important economically to the cattle industry. Recently, it has been determined that many helminthes (internal parasitic worms) have become increasingly resistant to current drug control strategies. Understanding the protective mechanisms is critical to develop new strategies to control internal parasites. ARS scientists in Beltsville, Maryland, characterized the transcriptome of parasite-resistant cattle using high-throughput technology. They identified cellular networks and biological pathways related to parasite resistance. In addition, three antimicrobials were identified that are likely involved in regulating host-parasite interactions. This information provides important insights into the immune regulation of host-parasite interactions and molecular mechanism of host resistance in cattle. This information facilitates applied breeding for parasite resistant animals and potentially vaccines that may confer protection against parasites.

Scientific Publication
Li, R.W., Chourdhary, R.K., Capuco, A.V., Urban Jr., J.F. 2012. Exploring the host transcriptome for mechanisms underlying protective immunity and resistance to helminth infection in ruminants. Vet. Parasit. 190(1-2):1-11.

Vaccinating Against Intestinal Parasites.
Anthelmintic resistance is a major problem in controlling parasites in production animals. Parasites produce proteins that modulate and suppress the host's immune responses providing an environment that is conducive to the parasite's survival. ARS scientists in Beltsville, Maryland, conducted a trial using a recombinant protein against the parasite, Ostertagia. The protein was used to vaccinate a small number of animals, which resulted in a high degree of protection against parasite infection and damage. Future studies using larger number of animals are being planned. In addition to the protein used in these studies, additional potential vaccine candidates have been identified. Developing vaccines against parasites will help reduce the reliance on drugs that are becoming increasing ineffective in controlling parasites.

Scientific Publication
Qu, G., Fetterer, R.H., Leng, L., Du, X., Zarlenga, D.S., Shen, Z., Han, W., Bucala, R., Tuo, W. 2014. Ostertagia ostertagi macrophage migration inhibition factor is present at all developmental stages and may cross-regulate host functions through host receptor. International Journal for Parasitology. 44(6):355-367.

Problem Statement 6B: Hemoparasitic Diseases

Babesiosis and Horses.
Equine piroplasmosis is a disease caused by blood parasites of the Babesia and Theileria families. It is considered a foreign animal disease in the United States and every effort has been made to prevent its entry into the U.S. horse population. The presence of this organism would

prove very expensive to the equine industry due to blocking of export and importation of horses. During 2010 the United States encountered the reemergence of piroplasmosis in the equine population. Piroplasmosis in horses is caused by two distinct parasites, Babesia equi (now Theileria equi) and Babesia caballi. In order to begin developing strategies to control and re-eliminate the organism from the U.S. equine population and in response to the needs of the USDA/APHIS, ARS scientists in Pullman, Washington, developed a method to eliminate persistent infection and transmission risk from horses infected with B. caballi. This has proven critical to the equine industry as it has resulted in owners of infected horses being able to treat their horses thus enabling them to resume their prior functions. In contrast, the second parasite T. equi, has proven more difficult to clear from infected horses. Research by scientists has resulted in the sequencing and annotating the genome of B. equi. It was discovered that this parasite is taxonomically between other Babesia organisms and a different blood borne parasite, Thieleria. The genetic information also determined that T. equi lacks a gene family that produces a classical antigenic variation. In addition, the ability of a native U.S. tick species, Amblyomma cajennense, was found to be an efficient vector for the parasite. This discovery will enable scientists to begin further research exploring and developing alternative intervention strategies to control this foreign disease entity in the U.S. equine population.

Scientific Publications

Scoles G.A., Hutcheson H.J, Schlater J.L., Hennager S.G., Pelzel A.M., Knowles D.P. 2011. Equine piroplasmosis associated with Amblyomma cajennense Ticks. Emerg Infect Dis. ct;17(10):1903-5.

Kappmeyer, L.S., Thiagarajan, M., Herndon, D.R., Ramsay, J.D., Caler, E., Djikeng, A., Gillespie, J.J., Lau, A.O., Roalson, E.H., Silva, J.C., Silva, M.G., Suarez, C.E., Ueti, M.W., Nene, V.M., Mealey, R.H., Knowles, D.P., Brayton, K.A. 2012. Comparative genomic analysis and phylogenetic position of Theileria equi. BMC Genomics. 9; 13:603.

Elimination of Persistent Infection and Transmission Risk Following the Re-emergence of Theileria equi in the United States.

Theileria equi is a tick-borne disease of horses that can cause severe acute disease characterized by fever, anemia, hemoglobinuria and in some cases death. Infected horses that recover from the acute disease become persistently infected for life. Disease caused by T. equi, called piroplasmosis, has been eradicated from the United States. In 2009, an outbreak of piroplasmosis occurred in Texas. Until recently, horses diagnosed with piroplasmosis were either euthanized or quarantined for life due to the persistence of infection. ARS scientists in Pullman, Washington, developed a treatment regimen using imidocarb dipropionate to eliminate T. equi from naturally infected horses and removed the risk of transmission of the pathogen to other horses. This allowed the horses to resume their previous lives and has facilitated international movement of horses between piroplasmosis infected countries and non-infected regions.

Scientific Publication

Ueti, M.W. Mealey, R.H., Kappmeyer, L.S., et al. 2012. Re-Emergence of the Apicomplexan Theileria equi in the United States: Elimination of Persistant Infection and Transmission Risk. PLOS ONE, 7(9), e44713.

Evaluation of the risk of indigenous ticks transmitting equine piroplasmosis.
Equine piroplasmosis was eradicated from the U.S. in the late 1980's. However, a recent outbreak in Texas caused significant economic loss to the equine industry and suggested that some ticks indigenous to the United States could play a role in transmission. ARS scientists in Pullman, Washington, in collaboration with Texas A&M University, collected and colonized ticks from horses at the outbreak ranch. The scientists demonstrated that these indigenous ticks were able to acquire and transmit the parasite to naïve horses. These results confirm that introduction of infected horses into areas of the United Staes containing competent indigenous vectors can result in dissemination of the parasite and thus disease to the equine population in the United States.

Scientific Publication
Scoles, G.A., Ueti, M.W. 2013. Amblyomma cajennense is an intrastadial biological vector of Theileria equi. Parasites and Vectors. Parasitesand vectors.com/content/6/1/306.

An Improved Diagnostic Test for Anaplasma marginale.
Anasplasma marginale, which causes anaplasmosis, is a tick-borne bacterial pathogen of cattle that causes economic losses to cattle industries throughout the world. A rapid, sensitive and specific diagnostic test is an essential tool for disease control within a herd. ARS scientists in Pullman, Washington, with collaborators from the Veterinary Medical Research and Development and Washington State University in Pullman, Washington, and the University of Idaho in Moscow, Idaho, developed an improved test to detect infected animals. This improved diagnostic test has a 99.7% specificity, increased from 97.8% specificity. The improved specificity means that fewer animals will be misdiagnosed as positive, when they are truly negative. This is of particular importance for herds that maintain an A. marginale free status.

Scientific Publication
Chung C, Wilson, C.B., Mudiyanselage, C.B., Kang, E., Adams, S.D., Kappmeyer, L.S., Knowles, Jr. D.P., Mcelwain, T., Evermann, J., Ueti, M.W., Scoles, G.A., Lee, S.S., Mcguire, T.C. 2014. Improved diagnostic performance of a commercial anaplasma antibody competitive enzyme-linked immunosorbent assay using recombinant major surface protein 5–glutathione S-transferase fusion protein as antigen. JVDI. 26(1):61-71.

Determination that a Fluorescently Marked Anaplasma marginale Strain was Attenuated in Cattle and Provided Protection against Disease upon Challenge with wild-Type A. marginale.
In general live, attenuated vaccines provide better protection against infection and disease than recombinant vaccines. In the case of A. marginale, the live, attenuated A. centrale is the only live vaccine available for use in some parts of the world to protect against anaplasmosis, a production limiting disease of cattle. However, even under the best circumstances, this live product produces only partial protection against clinical disease and must be produced in cattle, thus making it unavailable for use in the United States. ARS scientists in Pullman, Washington, with collaborators at Washington State University, determined that a live, fluorescently marked strain of A. marginale was attenuated in cattle and induced protective immunity upon challenge with wild-type A. marginale. This strain was grown in cell culture rather than in cattle, thus avoiding possible contamination with bovine blood and any associated pathogens. These are the first steps in development of a live, attenuated, vaccine that could be used to protect against

Anaplasmosis.

Scientific Publication
Hammac, G.K., Ku, P., Galletti, M.F., Noh, S.M., Scoles, G.A., Palmer, G.A., Brayton, K.A. 2013. Protective immunity induced by immunization with a live, cultured Anaplasma marginale strain. Vaccine. 31:3617-3622.

Component 7: Transmissible Spongiform Encephalopathies

Rationale for the research

Scrapie of sheep, bovine spongiform encephalopathy (BSE) of cattle, chronic wasting disease (CWD) of deer and elk, and variant Creutzfeldt-Jacob disease (vCJD) of humans are all fatal neurodegenerative disorders classified as transmissible spongiform encephalopathies (TSEs). There are no effective treatments or cure. The origin of TSEs has yet to be determined but scientific evidence indicates that the causal agents are abnormal prion proteins that induce a catalytic conversion of the normal host protein into an abnormal form. The abnormal prion proteins are transmissible and in most cases appear to be resistant to degradation. The discovery in 1996 that BSE of cattle is the cause of vCJD in people represented an unforeseen emerging zoonosis. That discovery has raised concern in the public health community that other TSEs such as CWD could evolve to cause disease in people. Although there is no evidence that CWD is zoonotic, TSEs have now been shown to be able to cross the species barrier, both experimentally and under natural conditions.

To date, only four cases of BSE have been found in the United States. The first case was found December 2003 in a cow imported from Canada and is estimated to have cost the U.S. beef industry $3.2 billion to $4.7 billion from the loss of beef and offal exports. The three subsequent cases were in cows born and raised in the United States. The first United States indigenous case was found in a downer cow in Texas, November 2004; the second indigenous cow was found on a farm in Alabama, March 2006; the third indigenous case was recently found on a dairy farm in California, April 2012. The finding of three indigenous cases of BSE, and the number of scrapie and CWD cases reported annually in the United States continues to raise concerns about the public health risks of animal TSEs.

Despite being caused by misfolding of a host encoded protein, it is now known that BSE exists as more than one strain. The form first identified has been termed classical BSE. This is the form associated with the feed-borne epizootic in the United Kingdom. More recently, two other forms have been identified and can be broadly referred to as atypical BSE, or specifically defined as High-type (H-type) or Low-type (L-type) BSE based upon their migration pattern relative to classical BSE on a Western blot. Neither H-type nor L-type BSE is associated with the feed-borne epizootic. Based on various forms of evidence, H-type and L-type BSE are generally believed to be spontaneous in nature rather than feed-borne, as is the case for classical BSE. The three indigenous U.S. BSE cases were reported as “atypical.” The first two indigenous BSE cases reported in 2004 and 2006 were reported as H-Type BSE, while the case in 2012 an L-type BSE.

ARS scientists have made significant contributions to the understanding of the atypical H-Type BSE cases found in the United States. ARS identified the first genetic case of BSE, and showed that it is a heritable polymorphism. ARS scientists have also contributed to the understanding of atypical BSE as a potential spontaneous TSE in cattle. This information supports, for the first time, the presence of three different etiologies (spontaneous/sporadic, genetic, and infectious/feedborne) of BSE in cattle. Previously, only humans were known to have three separate etiologies for TSEs. In collaboration with APHIS and a team of Italian researchers,

ARS has shown that the United States and Italian diagnostic techniques are equivalent in identifying classical, H-type, and L-type BSE, an important contribution that helped identify the April 2012 atypical L-type BSE case in California.

Scrapie, the TSE of sheep and goats, is the subject of an intensive eradication effort conducted by a federal-state-industry partnership. From FY 2003 through FY 2013, scrapie prevalence declined by 90 percent. The eradication program includes diagnostic testing using a platform and antibodies developed by ARS and a voluntary selective breeding program using data developed by ARS. Scrapie in goats is less well understood and ARS has contributed to improved diagnostics and basic studies on genetic resistance to TSEs of goats. Chronic wasting disease, the TSE of wild and captive deer and elk, is also monitored by live animal and post-mortem tests developed by ARS in collaboration with a network of wildlife agencies and university partners.

Stakeholders at the September 2011 Animal Health Planning Workshop representing the wildlife, sheep, and goat industries ranked TSE research as their 1st priority; representatives of the beef industry ranked TSE research as their 6th priority. Diseases in Component 7 include classical and atypical BSE, scrapie, and CWD.

Research Needs:
The Institute of Medicine of the National Academies published a guidance document November 2003 calling for a National Prion Research Program (Advancing Prion Science: Guidance for the National Prion Research Program – free download available from the National Academies Press at http://www.nap.edu/catalog.php?record_id=10862). Key recommendations from the National Academies report included funding basic research to elucidate: 1) the structural features of prions; 2) the molecular mechanisms of prion replication; 3) the mechanisms of pathogenesis of TSEs; and 4) the physiological function of the normal prion protein. In addition, the National Academies report recommended a comprehensive applied research program in diagnostics, testing blood for evidence of TSEs, epidemiological studies to monitor the occurrence of TSEs in human and animals, and research that will lead to strategies to prevent and treat TSEs.

The White House Office of Science and Technology Policy (OSTP) created a federal Interagency Working Group (IWG) on Prion Science in September 2004. ARS and the NIH co-led the IWG with participation Food and Drug Administration, APHIS, Centers for Disease Control and Prevention, Department of Defense, and Environmental Protection Agency. The working group determined that although significant scientific advances had been made, the research conducted to date had yet to deliver many of the concrete solutions needed to safeguard people and animals from these devastating diseases. A critical concern was the potential for environmental, genetic, or iatrogenic events that could lead to new variant TSEs that are infectious and zoonotic (transmissible from animals to humans). The following six priorities were selected by the IWG on Prion Science to maximize the impact of a National Prion Research Program:

- Nature and origin of prion agents
- Pathobiology of prion strains
- Determinants of transmissibility and epidemiology
- Genetics of disease susceptibility

- Diagnostics, detection, and surveillance
- Prevention and treatment

These six interrelated priorities represent areas with critical gaps in our knowledge base. They were selected with the aim of establishing strategic collaborations that will produce benefits by aligning core competencies across federal agencies.

Because TSE clinical studies in livestock and cervids require several years to reach an end-point, the associated expenses and resources needed to implement a research program, and the need for multidisciplinary research teams, ARS integrated its prion research laboratories located in Ames, Iowa, Pullman, Washington, and Albany, California, into a national coordinated research program in 2004. ARS focused its core competencies and the available resources of its national coordinated research program on six research needs: 1) understand infectivity, tissues tropism, and pathogenesis; 2) identify determinants of host range specificity; 3) understand the molecular mechanisms of prion replication; 4) strain characterization and determinants of virulence; 5) develop ante-mortem (live) pre-clinical animal tests; and 6) discover cost effective methods of prion inactivation. These research needs were the basis for determining the anticipated products that are expected from the research, and that now serve to help measure the national program's progress during the last five years in meeting the needs of animal producers, researchers, and action and regulatory agencies. The following list of anticipated products from the Action Plan is followed by the expected impact of the research and a sampling of relevant accomplishments.

Anticipated Products In Action Plan:

- Sensitive and specific ante-mortem tests that are rapid and scalable.
- Establish the biochemical, pathological, and epidemiological profile of atypical TSE strains and unusual isomers of the prion protein.
- Determine the pathogenesis of TSEs, including establishing route(s) of prion migration in the host, amplification of the agent, and disease expression.
- Conduct interspecies transmission studies to determine the host range specificity and resulting risk of TSEs to other animal species.
- Enhanced rapid methods of agent detection to protect the human environment.
- Cost effective methods of inactivating TSE agents.
- Identify and characterize genotypic variations and functional genomic mechanisms associated with disease susceptibility or resistance.

Impact:

The impact of the research included scientific information to enable regulatory and action agencies to promulgate science-based control programs. The development of diagnostics and countermeasures has enhanced current federal and state control and eradication programs for scrapie and CWD and will continue to enable the prevention and containment of future occurrences of BSE.

COMPONENT 7: SELECTED ACCOMPLISHMENTS

Problem Statement 7A: Nature and Origin of Prion Agents

Experimental Interspecies Transmission Studies of Transmissible Spongiform Encephalopathies in Cattle.

Experimental cross-species transmission of TSE agents provides valuable information for potential host ranges of known TSEs. Some interspecies transmission studies have been conducted by inoculating disease-causing prions intracerebrally (IC) rather than orally; the latter is generally effective in intraspecies transmission studies and is considered a natural route by which animals acquire TSEs. The "species barrier" concept for TSEs resulted from unsuccessful interspecies oral transmission attempts. Oral inoculation of prions mimics the natural disease pathogenesis route whereas IC inoculation is rather artificial; however, it is very efficient since it requires smaller dosage of inoculum, and typically results in higher attack rates and reduces incubation time compared to oral transmission. A species resistant to a TSE by IC inoculation would have negligible potential for successful oral transmission. To date, results indicate that cattle are susceptible to IC inoculation of scrapie, transmissible mink encephalopathy (TME), and CWD but it is only when inoculated with TME do they develop spongiform lesions or clinical disease similar to BSE. Importantly, cattle are resistant to oral transmission of scrapie or CWD; susceptibility of cattle to oral transmission of TME has not yet determined.

Scientific Publication

Hamir A.N., Kehrli M.E. Jr., Kunkle R.A., Greenlee J.J., Nicholson E.M., Richt J.A., Miller J.M., Cutlip R.C. 2011. Experimental interspecies transmission studies of the transmissible spongiform encephalopathies to cattle: comparison to bovine spongiform encephalopathy in cattle. J Vet Diagn Invest. 2011 May;23(3):407-20.

Experimental Transmission of Chronic Wasting Disease from Elk and White-tailed deer to Fallow Deer and Reindeer.

Final observations on experimental transmission of chronic wasting disease (CWD) from elk and white-tailed deer to fallow deer from a 5-year study were reported. During the study, 13 fawns were inoculated intracerebrally with CWD-infected brain material from white-tailed deer and 3 other fawns were kept as uninoculated controls. Animals were euthanized at 7, 24, 26, months post-inoculation (MPI), and between 29-37 and 51-60 MPI. Only five of the deer kept between 51 and 60 MPI became sick and were euthanized. Microscopic lesions of spongiform encephalopathy were observed in only these five animals; however, abnormal prions were detected in tissues of the central nervous system by immunohistochemistry, Western blot, and by a commercial rapid test in all animals that survived beyond 24 months post-infection. This study demonstrated that intracerebrally inoculated fallow deer not only amplify CWD prions, but also develop lesions of spongiform encephalopathy. These results provide information on the potential risk of CWD transmission across different cervid species, and contributes to understanding CWD transmission in wild and captive cervids.

CWD in reindeer is a serious threat to the livelihood and cultural integrity of indigenous peoples of the northern region of North America. The susceptibility of the species was established through an oral challenge, representing the presumed natural route of infection in the wild.

Antemortem diagnosis and postmortem confirmatory testing demonstrated that two of three reindeer challenged with white tailed deer CWD developed the disease within 2 years. The third reindeer in this group, and 3 reindeer orally inoculated with CWD of elk origin, failed to develop CWD. These 4 animals all had a polymorphism in the prion gene encoding a novel change at position 138. These findings demonstrate that (i) a sub-population of reindeer are susceptible to CWD by oral inoculation implicating the potential for transmission to other Rangifer species, and (ii) certain reindeer PRNP polymorphisms may be protective against CWD infection.

Scientific Publications

Hamir A.N., Greenlee J.J., Nicholson E.M., Kunkle R.A., Richt J.A., Miller J.M., Hall M. 2011. Experimental transmission of chronic wasting disease (CWD) from elk and white-tailed deer to fallow deer by intracerebral route: final report. Can J Vet Res. 2011 Apr;75(2):152-6.

Mitchell, G.B., Sigurdson, C.J., O'Rourke, K.I., Algire, J., Harrington, N.P., Walther, I., Spraker, T.R., Balachandran, A. 2012. Experimental oral transmission of chronic wasting disease to reindeer (Rangifer tarandus tarandus). PLoS One. 7:e39055.

Problem Statement 7B: Pathobiology of Prion Strains

Stability Profiling of Typical and Atypical Transmissible Spongiform Encephalopathy (TSE) Isolates.

Scientists at the National Animal Disease Center, Ames, Iowa, developed a method for the rapid evaluation of the stability of the disease-associated form of the prion protein (PrPSc). This method was applied to isolates of sheep scrapie and bovine spongiform encephalopathy (BSE). Comparisons between isolates and hosts with different genotypes were evaluated for both sheep and cattle. For sheep scrapie, results showed that the stability of PrPSc correlates with the disease incubation time in the animal and that the process known as strain stabilization, whereby a TSE is passed serially through a host species, does not alter the physical properties of the infectious agent. Thus, changes in disease incubation time that are known to occur are likely due to changes such as the infectious dose of the inoculating material. For BSE, ARS scientists showed that the stability of cattle PrPSc, as defined by resistance to denaturant unfolding of the fibrils, is largely invariant with the exception of atypical H-type BSE, which exhibits a higher stability. The stability of a genetic form of BSE (E211K BSE) is consistent with its previous assignment as an H-type strain. In addition to the increased knowledge with regard to strains of scrapie in sheep and BSE in cattle, the methodologies used here can also be applied to research on other species (such as goats, deer, and elk) to improve our understanding of the properties of TSE strains of scrapie, BSE, and CWD in natural hosts.

Scientific publications

Vrentas, C.E., Greenlee, J.J., Tatum, T.L., Nicholson, E.M. 2012. Relationships between PrPSc stability and incubation time for United States scrapie isolates in a natural host system. PLoS ONE. 7(8):e4306

Vrentas, C.E., Greenlee, J.J., Baron, T., Caramelli, M., Czub, S., Nicholson, E.M. Stability properties of PrPSc from cattle with experimental transmissible spongiform encephalopathies. BMC Vet Res. 2013; 9: 167.

Clinical and Pathologic Features of a Genetic Case of BSE.
The majority of bovine spongiform encephalopathy (BSE) cases have been ascribed to the classical form of the disease. H-type and L-type BSE cases have atypical molecular profiles relative to classical BSE and are thought to arise spontaneously. However, one case of H-type BSE was associated with a heritable E211K mutation in the prion protein gene. Scientists at the National Animal Disease Center, Ames, Iowa, conducted a study to evaluate the transmission of this unique isolate of H-type BSE when inoculated into a calf of the same genotype by the intracranial route. Electroretinograms were used to demonstrate preclinical deficits in retinal function and optical coherence tomography was used to demonstrate an antemortem decrease in retinal thickness. The calf rapidly progressed to clinical disease (9.4 months). Widespread distribution of abnormal prion protein was demonstrated within neural tissues by western blot and immunohistochemistry. While this isolate is categorized as BSE-H due to a higher molecular mass of the unglycosylated PrPSc isoform, differences in the specific western blot pattern indicate it is unique from other described cases of BSE-H. This work demonstrates that this isolate is transmissible, has a BSE-H phenotype when transmitted to cattle with the K211 polymorphism, and has molecular features that distinguish it from other cases of BSE-H described in the literature.

Scientific publication
Greenlee, J.J., Smith, J.D., West Greenlee, M.H., Nicholson, E.M. Clinical and pathologic features of H-type bovine spongiform encephalopathy associated with E211K prion protein polymorphism. PLoS One. 2012;7(6):e38678.

Problem Statement 7C: Determinants of Transmissibility and Epidemiology

Accumulation of Prion Scrapie in the Placentas of Goats.
Domestic goats are a natural and experimental host of scrapie and bovine spongiform encephalopathy. Goats are also susceptible to experimental infection with Chronic Wasting Disease and Creutzfeldt Jakob disease. Distribution of prion scrapie is similar in the tissues of scrapie-infected sheep and goats but no data are available on the potential shedding of the agent through the placenta, the presumed route of transmission of ovine scrapie. ARS scientists in Pullman, Washington, studied the accumulation of prion scrapie in the placentas of goats with naturally acquired classical scrapie in comparison to field cases of classical ovine scrapie. The results of these studies showed that prion scrapie accumulates in the shed placentas of goats with naturally acquired scrapie. Although these levels were low in most caprine samples, the caprine placenta may contribute to prion contamination of kidding facilities and transmission to co-housed sheep or goats.

Scientific Publication
O'Rourke K.I., Zhuang D., Truscott T.C., Yan H., Schneider D.A. 2011. Sparse PrP(Sc) accumulation in the placentas of goats with naturally acquired scrapie. BMC Vet Res.1; 7:7.

Problem Statement 7D: Genetics of Prion Disease Susceptibility

Pathologic and Biochemical Characterization of a Genetic Gorm of Bovine Spongiform Encephalopathy (BSE).
Transmissible spongiform encephalopathies (TSE) such as BSE are characterized by a novel transmissible "infectious" protein called a prion that converts the cellular form of the prion protein (PrPc), normally expressed by many cells in the body, to a misfolded, disease-associated form (PrPd) that causes pathological lesions in the central nervous system. The complete pathologic and biochemical features of a genetic form of BSE were defined and reported for the first time by ARS scientists at the National Animal Disease Center, Ames, Iowa. The genetic form of BSE is analogous to the most prevalent hereditary form of human TSE. Heritable BSE along with spontaneous BSE forms are also referred to as atypical BSE cases which have important implications in that they are not associated with the feedborne epidemic of classical BSE first recognized in the United Kingdom in the 1980s. Atypical BSE cases emphasize the need to maintain the specified risk material ruminant feed ban as a science-based policy to prevent a feedborne epidemic from developing; the feedborne nature of the classical BSE epidemic has been demonstrated to negatively impact export markets in various countries around the world, whereas atypical BSE does not connote the same concern.

Scientific Publication
Greenlee, J.J., Smith, J.D., West Greenlee, M.H., Nicholson, E.M. 2012. Clinical and pathologic features of H-type bovine spongiform encephalopathy associated with E211K prion protein polymorphism. PLoS ONE. 7(6):e38678.

A Genetic Marker Associated with Resistance to Scrapie.
The amino acid, lysine, at position 171 of the sheep prion protein delays development of scrapie. ARS scientists demonstrated the effect of the amino acid lysine at position 171 of the sheep prion protein on susceptibility to scrapie, a transmissible spongiform encephalopathy of sheep. Amino acid differences in the prion protein are known to play a major role in scrapie susceptibility in sheep and these genetic differences are utilized in the strategy to remove scrapie from our nation's sheep flock. Natural scrapie had previously only been described in one sheep with lysine at position 171 of the prion protein, hence not enough information was available from natural cases to determine the effect of lysine at position 171 on scrapie susceptibility. ARS scientists at the National Animal Disease Center, Ames, Iowa, demonstrated that sheep with a prion protein containing lysine at position 171 are susceptible to scrapie but have a prolonged scrapie incubation period, and that the abnormal prion protein accumulates throughout the central nervous system and lymphoid organs. Because sheep with lysine at prion amino acid position 171 develop scrapie at a slower rate than other known susceptible genotypes this information is critical to sheep breeders that want to eradicate genotypes susceptible to scrapie.

Scientific Publication
Greenlee, J.J., Zhang, Xia, Nicholson, E.M., Kunkle, R.A., Hamir, A.N. 2012. Prolonged incubation time in sheep with prion protein containing lysine at position 171. Journal of Veterinary Diagnostic Investigation. 24(3):554-558.

Prolonged Scrapie Incubation in Goats Linked to Two Genetic Markers.
ARS scientists performed the first oral scrapie challenge of goats heterozygous for two PrPc polymorphisms (commonly referred to as genetic variation or alleles) of particular interest to scrapie susceptibility. Scrapie eradication in sheep is based in part on strong genetic resistance to classical scrapie. Goats may serve as a scrapie reservoir but there has been limited experimental inoculation to confirm strong genetic resistance in goats. The results confirmed that goats singly heterozygous at the two PrPc alleles (S142 or K222) have greatly extended incubation times, indicating a need in scrapie-eradication programs for longer trace-back histories for goats bearing these alleles. Also indicated is a need to assess goats singly homozygous for either of these alleles for enhanced resistance to scrapie infection.

Scientific Publication
White, S.N., Reynolds, J.O., Waldron, D.F., Schneider, D.A. & O'Rourke, K.I. 2012. Extended scrapie incubation time in goats singly heterozygous for PRNP S146 or K222. Gene 501:49-51.

Problem Statement 7E: Diagnostics, Detection, and Surveillance

Development of a Rapid Method for Detection of Disease-associated Prions.
A method for the detection of abnormal prions (PrPSc) in formalin-fixed paraffin-embedded tissue by ELISA has been developed and described by ARS scientists at the National Animal Disease Center, Ames, Iowa. Methods for diagnosis of transmissible spongiform encephalopathies (TSEs) in cattle, sheep and cervids have traditionally depended on the availability of both frozen fresh and formalin-fixed tissues. However, in many diagnostic sample submissions only formalin-fixed samples have been available for TSE diagnosis, a situation that previously precluded analysis by rapid diagnostic procedures such as ELISA. This work describes a method suitable for extraction of the PrPSc from formalin-fixed paraffin-embedded tissue for detection by ELISA. This represents a significant advancement for diagnostic laboratories and provides a rapid alternative method for TSE detection beyond immunohistochemistry (IHC).

Scientific Publication
Nicholson, E.M., Greenlee, J.J., Hamir, A.N. 2011. PrPSc detection in formalin-fixed paraffin-embedded tissue by ELISA. BMC Research Notes. 4(1):432.

Control of Chronic Wasting Disease (CWD) in Deer and Elk through Live Animal Diagnosis and Genetic Selection.
Elk and deer are farmed in many parts of the United States. As CWD in the wild population continues to spread, the ability to monitor the disease in free ranging cervids and perform surveillance on farmed deer and elk are critical for the survival of the captive cervid industry. Using the test platform developed for sheep, federal and state laboratories perform tissue based testing on clinical suspects, slaughter samples, and hunter harvested deer and elk. Coupled with an understanding of the role of prion genotypes in prolonging incubation time, the regulatory groups have an arsenal of methods for CWD management suitable in their regions.

Scientific publications

Monello, R.J., Powers, J.G., Hobbs, N.T., Spraker, T.R., O'Rourke, K.I., Wild, M.A. 2013. Efficacy of antemortem rectal biopsies to diagnose and estimate prevalence of chronic wasting disease in free-ranging cow elk (Cervus elaphus nelsoni). Journal of Wildlife Diseases. 49:270-278.

Thomsen, B.V., Schneider, D.A., O'Rourke, K.I., Gidlewski, T., McLane, J., Allen, R.W., McIsaac, A.A., Mitchell, G.B., Keane, D.P., Spraker, T.R., Balachandran, A. 2012. Diagnostic accuracy of rectal mucosa biopsy testing for chronic wasting disease within white-tailed deer (Odocoileus virginianus) herds in North America: Effects of age, sex, polymorphism at PRNP codon 96, and disease progression. Journal of Veterinary Diagnostic Investigation. 24:878-887.

Confirmatory Laboratory Tests to Detect Classical and Atypical BSE Forms.
ARS scientists in Ames, Iowa, obtained brain samples from cases of United States and Italian Classical (C-) type, U.S. High (H-) type, and an Italian Low (L-) type BSE to compare the ability of two sets of immunohistochemical (IHC) and Western blot (WB) confirmatory protocols to detect C- and atypical (L- and H-type) BSE forms. The study showed that the IHC and WB BSE confirmatory protocols were equally able to recognize C-, L- and H-type BSE forms and to discriminate between their different immunohistochemical and molecular phenotypes. Importantly, for the first time, one of the two sets of BSE confirmatory protocols proved effective in identifying the L-type BSE form. This finding helped validate the suitability of the BSE confirmatory tests for BSE surveillance currently in place in the United States.

Scientific Publication
Porcario C., Hall SM, Martucci F., Corona C., Iulini B., Perazzini A.Z., Acutis P., Hamir A.N., Loiacono C.M., Greenlee J.J., Richt J.A., Caramelli M., Casalone C. 2011. Evaluation of two sets of immunohistochemical and Western blot confirmatory methods in the detection of typical and atypical BSE cases. BMC Res Notes. 2011 Sep 29;4:376.

Prion Infectivity in Scrapie-infected Sheep and Goat Blood.
ARS researchers at the Animal Disease Research Unit in Pullman, Washington, have identified the components of sheep's and goat's blood that carry prion infectivity by using the sensitive technique of transfusion bioassay. The presence of infectious scrapie prions in the blood indicates the possibility of developing a blood-based diagnostic test but currently available immunoassays do not appear to be sensitive enough for robust detection in samples of whole blood. The insights gained are an important step toward optimizing the isolation of the blood components most relevant to early disease detection by immunoassay in both sheep and goats.

Scientific Publications
Dassanayake R.P., Schneider D.A., Truscott T.C., Young A.J., Zhuang D, ORourke K.I. 2011 Classical scrapie prions in ovine blood are associated with B lymphocytes and platelet rich plasma. BioMed Central (BMC) Veterinary Research. 7:75.

Dassanayake, R.P., Schneider, D.A., Herrmann-Hoesing, L.M., Truscott, T.C., Davis, W.C., O'Rourke, K.I. 2012. Cell-surface expression of PrPC and the presence of scrapie prions in the blood of goats. J Gen Virol. 93(5):1127-1131.

Detection of Disease Associated Prion Protein in Retina of Sheep and Cattle using a Commercially Available Diagnostic Kit.
Scientists from the National Animal Disease Center, Ames, Iowa, evaluated samples from experimental animal challenge studies to assess the usefulness of retina samples for detection of prion positive animals, using a commercially available enzyme immunoassay (EIA) intended for rapid identification of sheep and cattle with transmissible spongiform encephalopathies (TSEs). Retina sample EIA results were in agreement with results of brainstem sample EIA or confirmatory assay results for negative control animals and TSE-inoculated animals with clinical signs of disease. However, TSE-inoculated animals with positive confirmatory assay results that did not have clinical signs of disease had negative retina sample EIA results. Retina sample EIA results were in agreement with brainstem sample immunohistochemistry results for 4 TSE-inoculated sheep with negative retropharyngeal lymph node EIA results. Results from this study suggest that retina samples may be useful for rapid EIA screening of animals with neurologic signs to detect TSEs.

Scientific publication
Smith, J.D., Greenlee, J.J. Detection of misfolded prion protein in retina samples of sheep and cattle by use of a commercially available enzyme immunoassay. Am J Vet Res. 2014; 75(3): 268-72.

Problem Statement 7F: Prevention and Treatment

Methods for Inactivation of the TSE Agent
Prions demonstrate an unusual resistance to methods effective at inactivating conventional microorganisms, and the only means by which successful inactivation can be validated is by a bioassay in animals. The difficulty in inactivation and in developing methods to test and validate the inactivation of TSE agents has resulted in a very tangible and difficult challenge for the medical and veterinary communities, as well as animal agriculture and related industries. To address both problems, ARS scientists at the National Animal Disease Center, Ames, Iowa, have evaluated a means by which the harsh chemical treatment to inactivate prions can be removed prior to conducting the bioassay, and they also evaluated milder treatments in a combinatorial approach. Through these efforts, acetone was shown to suitably precipitate misfolding prion proteins (PrPSc), allowing the inoculation of animals for bioassay without the large dilution of infectivity usually required to dilute harsh chemicals before the test material can be inoculated in animals. This method now allows researchers to more readily assess inactivation approaches, providing an important tool for further study of TSE agent inactivation. The assessment of milder treatments used in combination showed that such an approach can markedly reduce TSE infectivity in a manner greater than individual treatments alone. This indicated that suitable approaches for TSE agent inactivation may be developed using combinations of treatments that are alone not sufficient to inactivate the TSE agent.

Scientific publications
Smith, J.D., Nicholson, E.M., Greenlee, J.J. Evaluation of a combinatorial approach to prion inactivation using an oxidizing agent, SDS, and proteinase K. BMC Vet Res. 2013; 9: 151.

Smith, J.D., Greenlee, J.J., Foster, G.H., and Nicholson, E.M. Acetone precipitation of the scrapie agent results in successful recovery of PrPSc but decreased infectivity. J Agric Food Chem. 2012; 60: 4758-4762.

Appendices

APPENDIX 1 – *Research Projects in National Program 103*
APPENDIX 2 – *Publications by Research Project*
APPENDIX 3 – *ARS Technology Transfer*
APPENDIX 4 – *Research Collaborations*
APPENDIX 5 – *Retrospective Electronic Survey Results*

APPENDIX 1

National Program 103 – Animal Health ACCOMPLISHMENT REPORT 2011 – 2015 List of Research Projects

Albany, California Western Regional Research Center Produce Safety and Microbiology Research Unit	
2030-32000-009-00D	Immunodiagnostics to Detect Prions and Other Important Animal Pathogens
Ames, Iowa National Animal Disease Center Infectious Bacterial Diseases Research Unit	
5030-32000-104-00D	Prevention and Control Strategies for Tuberculosis in Cattle and Wildlife Reservoirs
5030-32000-107-00D	Control, Immunology, and Genomics of Spirochete Diseases
5030-32000-110-00D	Immunology and Intervention Strategies for Johne's Disease
5030-32000-111-00D	Identification of Disease Mechanisms and Development of Improved Diagnostics and Vaccines for Brucellosis in Livestock and Wildlife
National Animal Disease Center Ruminant Diseases and Immunology Research Unit	
5030-32000-102-00D	Identification of Host Immune Factors and Intervention Strategies for Mastitis
5030-32000-105-00D	Identification of Disease Mechanisms and Control Strategies for Bacterial Respiratory Pathogens in Cattle
5030-32000-106-00D	Intervention Strategies to Control Viral Diseases of Cattle
3625-32000-005-00D	Metabolic and Infectious Disease Resistance
3625-32000-079-00D	Characterization and Enhancement of Immune Responses of Calves
3625-32000-085-00D	Molecular, Nutrient, and Endocrine Basis of Metabolic Diseases that Affect the Reproductive Health of the Diary Cow
National Animal Disease Center Virus and Prion Research Unit	
5030-32000-103-00D	Transmission, Differentiation, and Pathobiology of Transmissible Spongiform Encephalopathies
5030-32000-108-00D	Intervention Strategies to Control Viral Diseases of Swine
5030-32000-109-00D	Strategies to Control and Prevent Bacterial Infections in Swine
3625-32000-092-00D	Identification of New Pathogens and Predictors of Emerging Livestock Diseases
Athens, Georgia Southeast Poultry Research Laboratory Endemic Poultry Viral Diseases Research Unit	
6040-32000-065-00D	Genomic Strategies for Control of Herpesviruses of Poultry
6040-32000-067-00D	Invention Strategies to Control and Prevent Enteric Viral Diseases of Poultry

Southeast Poultry Research Laboratory Exotic and Emerging Avian Viral Diseases Research Unit	
6040-32000-062-00D	Characterization of Protective Host Responses to Avian Influenza Virus Infections in Avian Species
6040-32000-063-00D	Intervention Strategies to Control and Prevent Disease Outbreaks Caused by Avian Influenza and Other Emerging Poultry Pathogens
6040-32000-064-00D	Intervention Strategies to Control Newcastle Disease
Beltsville, Maryland Animal Biosciences & Biotechnology Laboratory	
8042-32000-097-00D	Functional Genomics Approaches for Controlling Diseases of Poultry
Animal Genomics and Improvement Laboratory	
8042-32000-093-00D	Development of Genomic Tools to Study Ruminant Resistance to Gastrointestinal Nematodes
8042-32000-092-00D	Novel Intervention Strategies and Genomics for Controlling Mastitis
Animal Parasitic Diseases Laboratory	
8042-31320-076-00D	Development of Control and Intervention Strategies for Avian Coccidiosis
8042-32000-094-00D	Immunological Approaches to Controlling Swine Intestinal Parasites and Mucosal Pathogens
8042-32000-095-00D	Parasitic Biodiversity and the U.S. National Parasite Collection
8042-32000-096-00D	Molecular and Immunological Approaches to Controlling GI Nematode Infections of Ruminants
8042-32000-098-00D	Functional Genomics Approaches for Controlling Diseases of Swine
Clay Center, Nebraska U.S. Meat Animal Research Center Genetics, Breeding, and Animal Health Research	
3040-32000-031-00D	Genetic and Biological Determinants of Respiratory Diseases of Ruminants
East Lansing, Michigan Avian Disease and Oncology Research Laboratory	
5050-32000-016-00D	Genetic and Biological Determinants of Avian Tumor Virus Pathogenicity, Transmission, and Evolution
Fayetteville, Arkansas Poultry Production and Products Safety Research	
6022-32000-012-00D	Novel Therapeutic, Diagnostic, and Management Strategies to Reduce Antibiotic Use in Poultry Production
Manhattan, Kansas Center for Grain and Animal Health Research Arthropod-Borne Animal Diseases Research Unit	
3020-32000-005-00D	Countermeasures to Prevent, Mitigate, and Control Rift Valley Fever (RVF)
3020-32000-006-00D	Bluetongue Virus Pathogenesis, Epidemiology, and Control Measures
3020-32000-008-00D	Predictive Biology of Emerging Vector-Borne Viral Diseases
5410-32000-016-00D	Molecular Biology and Pathogenesis of Arboviruses
Mississippi State, Mississippi Poultry Research	
6064-32000-011-00D	Strategies to Control and Prevent Avian Mycoplasmosis
6406-32000-005-00D	Diagnosis and Control of Mycoplasmosis in Poultry

Orient Point, New York Plum Island Animal Disease Center	
8064-32000-056-00D	Countermeasures to Control Foreign Animal Diseases of Swine
8064-32000-057-00D	Intervention Strategies to Support the Global Control and Eradication of Foot-and-Mouth Disease Virus (FMDV)
8064-32000-058-00D	Ecology and Pathogenesis of Re-Emerging Vesicular Stomatitis Virus (VSV) in North America
1940-32000-055-00D	Advanced Vaccines for Foot-and-Mouth Disease and Classical Swine Fever
Pullman, Washington Animal Disease Research Unit	
2090-32000-030-00D	Mitigating the Risk of Transmission and Environmental Contamination of Transmissible Spongiform Encephalopathies
2090-32000-031-00D	Control of Ovine Respiratory Disease through Genetic and Immunologic Mitigation of Pathogen Transmission and Disease
2090-32000-032-00D	Immunological Intervention of Malignant Catarrhal Fever Virus-Induced Disease in Ruminants
2090-32000-033-00D	Development of Strategies to Control Anaplasmosis
2090-32000-034-00D	Pharmacological and Immunologic Interventions against Vector-Borne Bovine and Equine Babesiosis

APPENDIX 2

National Program 103 – Animal Health
ACCOMPLISHMENT REPORT 2011 – 2015
Publications by Location and Research Project

Albany, California
Western Regional Research Center
Produce Safety and Microbiology Research Unit

2030-32000-009-00D
Immunodiagnostics to Detect Prions and Other Important Animal Pathogens

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Ames, Iowa

National Animal Disease Center

Infectious Bacterial Diseases Research Unit

5030-32000-104-00D

Prevention and Control Strategies for Tuberculosis in Cattle and Wildlife Reservoirs

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Palmer, M.V., Thacker, T.C., Waters, W.R., Robbe-Austerman, S. 2014. Oral vaccination of white-tailed deer (Odocoileus virginianus) with Mycobacterium bovis Bacillus Calmette-Guerin (BCG). PLoS One. 9(5):e97031.

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5030-32000-107-00D

Control, Immunology, and Genomics of Spirochete Diseases

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Immunology and Intervention Strategies for Johne's Disease

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National Animal Disease Center

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Identification of Host Immune Factors and Intervention Strategies for Mastitis

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Strategies to Control and Prevent Bacterial Infections in Swine

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Identification of New Pathogens and Predictors of Emerging Livestock Diseases

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Invention Strategies to Control and Prevent Enteric Viral Diseases of Poultry

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Characterization of Protective Host Responses to Avian Influenza Virus Infections in Avian Species

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Animal Genomics and Improvement Laboratory

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Development of Genomic Tools to Study Ruminant Resistance to Gastrointestinal Nematodes

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Novel Intervention Strategies and Genomics for Controlling Mastitis

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Animal Parasitic Diseases Laboratory

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Development of Control and Intervention Strategies for Avian Coccidiosis

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Immunological Approaches to Controlling Swine Intestinal Parasites and Mucosal Pathogens

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Parasitic Biodiversity and the U.S. National Parasite Collection

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Functional Genomics Approaches for Controlling Diseases of Swine

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Genetic and Biological Determinants of Respiratory Diseases of Ruminants

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Genetic and Biological Determinants of Avian Tumor Virus Pathogenicity, Transmission, and Evolution

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Fayetteville, Arkansas

Poultry Production and Products Safety Research

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Novel Therapeutic, Diagnostic, and Management Strategies to Reduce Antibiotic Use in Poultry Production

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Countermeasures to Prevent, Mitigate, and Control Rift Valley Fever (RVF)

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Poultry Research

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Strategies to Control and Prevent Avian Mycoplasmosis

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Countermesasures to Control Foreign Animal Diseases of Swine

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Intervention Strategies to Support the Global Control and Eradication of Foot-and-Mouth Disease Virus (FMDV)

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Ecology and Pathogenesis of Re-Emerging Vesicular Stomatitis Virus (VSV) in North America

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Animal Disease Research Unit

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Mitigating the Risk of Transmission and Environmental Contamination of Transmissible Spongiform Encephalopathies

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Control of Ovine Respiratory Disease through Genetic and Immunologic Mitigation of Pathogen Transmission and Disease

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Immunological Intervention of Malignant Catarrhal Fever Virus-Induced Disease in Ruminants

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Development of Strategies to Control Anaplasmosis

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Pharmacological and Immunologic Interventions against Vector-Borne Bovine and Equine Babesiosis

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APPENDIX 3

National Program 103 – Animal Health
ACCOMPLISHMENT REPORT 2011 – 2015

Technology Transfer

Over the last two 5-year cycles of NP 103, the following highlight NP 103 technology transfer outcomes:

Type	2007-2011	2011-2015
Inventions	62	65
Patents filed	19	34
Patents approved	7	8
Biological material inventions	30	8
Biological material licenses from inventions	20	8

APPENDIX 4

National Program 103 – Animal Health
ACCOMPLISHMENT REPORT 2011 – 2015

Research Collaborations

Number of Cooperative Research and Development Agreements (CRADAs): 29
Number of Trust Funds: 129
Number of Reimbursables: 305

External Grant Sources of Funding for National Program 103 Projects 2011-2015

Grants from universities usually involved cooperative research projects jointly conducted with university partners. In many cases this funding originated from third parties, such as the USDA National Institute for Food and Agriculture, National Institutes of Health, USAID, National Science Foundation, and other industry, non-profit, and government sources.

University	Government	International	Industry	Misc
23	63	26	47	30

Universities:
Chung-Ang University, Korea
Clemson University
Iowa State University
Kansas State University
Northern Arizona University
Oklahoma State University
Pennsylvania State University
Rutgers University
South Dakota State University
University of Alberta, Canada
University of Calgary, Canada
University of California
University of Cambridge, United Kingdom
University of Connecticut
University of Georgia
University of Iowa
University of Massachusetts
University of Minnesota
University of Nebraska
Washington University

Companies:
Agtech Products
Amlan International
Arkion Life Sciences
Aviagen North America, Inc.
Axiss France SAS - Pancosma Bioactives
Biomune Company
Boehringer Ingelheim Animal Health
Cattle Stats, LLC
Chesapeake Perl, Inc.
Choong Ang Vaccine Lab
Cobb-Vantress, Inc.
Elanco Animal Health
Genome Alberta
Guardian Biotechnologies
Harrisvaccines, Inc.
IDEXX Laboratories, Inc.
Investigacion Aplicada, S.A. de C.V.
Laboratories of C.V. Avilab S.A.
Medigen, Inc.
Merial
Merck and Company

Companies cont:
Naturence Co. Ltd.
Novartis Animal Health US, Inc.
Novozymes A/S
Pacific Genetech Limited
Pancosma S.A.
Pfizer Animal Health, Pfizer, Inc.
Piggen Canada, Inc.
Prionics Ag
Prosetta Bioconformatics, Inc.
Rural Technologies, Inc.
Seppic
Veterinary Medical Research Development (VMRD)
Viridax Corporation
Vital Probes, Inc.
Zoetis

Research Organizations:
International Livestock Research Institute
Teagasc, Ireland
U.S.-Israel Binational Science Foundation

Industry:
American Egg Board
National Cattlemen's Beef Associaion
National Pork Board
U.S. Poultry and Egg Association
USA Poultry & Egg Export Council

Government:
Canadian Food Inspection Agency
Centers for Disease Control and Prevention, Department of Health and Human Services
Defense Threat Reduction Agency
Environmental Protection Agency
National Institute of Allergy and Infectious Diseases, Department of Health and Human Services
National Institutes of Health, Department Of Health and Human Services
National Veterinary Research & Quarantine Service (NVRQS), Republic of Korea
Office of Naval Research
U.S. Agency for International Development (USAID)

Government cont:
U.S. Department of Defense
U.S. Department Of Homeland Security
U.S. Department of State
U.S. Geological Survey, Department of the Interior
USDA Foreign Agricultural Service (FAS)
USDA National Institute of Food and Agriculture (NIFA)

Non-Government Organization
Biotechnology Research and Development Center (BRDC)
Commonwealth Scientific and Industrial Research Organisation (CSIRO)
Food and Agricultural Organization of the United Nations
GALVMED (Global Alliance for Livestock Veterinary Medicines)
Institute for Animal Health
Kansas Bioscience Authority
U.S.-Israel Binational Agricultural R&D Fund (BARD)

Outgoing Funding to Support ARS Research Programs
2011-2015
Specific Cooperative Agreements

United States Universities
Auburn University
Colorado State University
Georgia Southern University
Iowa State University
Kansas State University
Michigan State University
Mississippi State University
Ohio State University
South Dakota State University
Stonybrook University
Texas A&M University
University of California
University of Connecticut
University of Georgia
University of Idaho
University of Maryland
University of Minnesota
University of Missouri
University of Vermont and State Agricultural College
University of Washington
University of Wisconsin
University of Wyoming
University Texas Medical Branch
Virginia Commonwealth Unit
Virginia Polytechnic Institute and State University
Washington State University
Washington University

International Universities
Ben-Gurion University of the NEGV, Israel
Federal University of Santa Maria, Brazil
Makerere University, Uganda
Novosibirsk State University, Russia
Simon Fraser University, Canada
University of Cambridge, United Kingdom
University of Copenhagen, Denmark
University of Dodoma, Tanzania
University of the Basque Country, Spain
Warsaw University of Life Sciences, Poland

U.S. Government

Smithsonian Institution

International Research Organizations
Animal Sciences Institute, National Agricultural Research Center, Pakistan
Canadian Food Inspection Agency, Canada
Harbin Veterinary Research Institute, China
INIA (Instituto Nacional de Investigacion y Tecnologia y Alimentaria, Spain
Indian Council of Agricutltural Research, India
Instituto Nacional de Tecnologia Agro, Argentina
International Centre of Insect Physiology, Kenya
International Livestock Research Institute, Kenya
Kenya Agricultural Research Institute, Kenya
Kenya Department of Veterinary Services, Kenya
Ministry of Agriculture of the Russian Federation, Russia
National Lab for Quality Control, Egypt
National Veterinary Laboratory, Cameroon
Philippine Animal Health Center, Philippines
Vietnam Department of Animal Health, Vietnam

U.S. Companies
Floragenex, Inc.
Green Mountain Antibodies, Inc.
Kingfisher Biotech, Inc.

International Companies
Sagarpa-Senasica, Mexic

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APPENDIX 5

National Program 103 – Animal Health
ACCOMPLISHMENT REPORT 2007 – 2011

2011 Stakeholder Survey PowerPoint

National Program Assessment
Electronic Stakeholder Survey
Animal Health National Program

September 2011

Cyril Gerard Gay, DVM, Ph.D
Senior National Program Leader
Animal Production and Protection

Purpose

- Conduct a “National” Program Assessment
- Obtain genuine, authentic, factual information
- Assess program in a cost effective manner
- Assess whether the national program had impact
- Stakeholders and partners:
  - Producers and Farmers
  - University scientists
  - Industry
  - Trade associations
  - Scientific associations
  - Federal government agencies
  - State government agencies

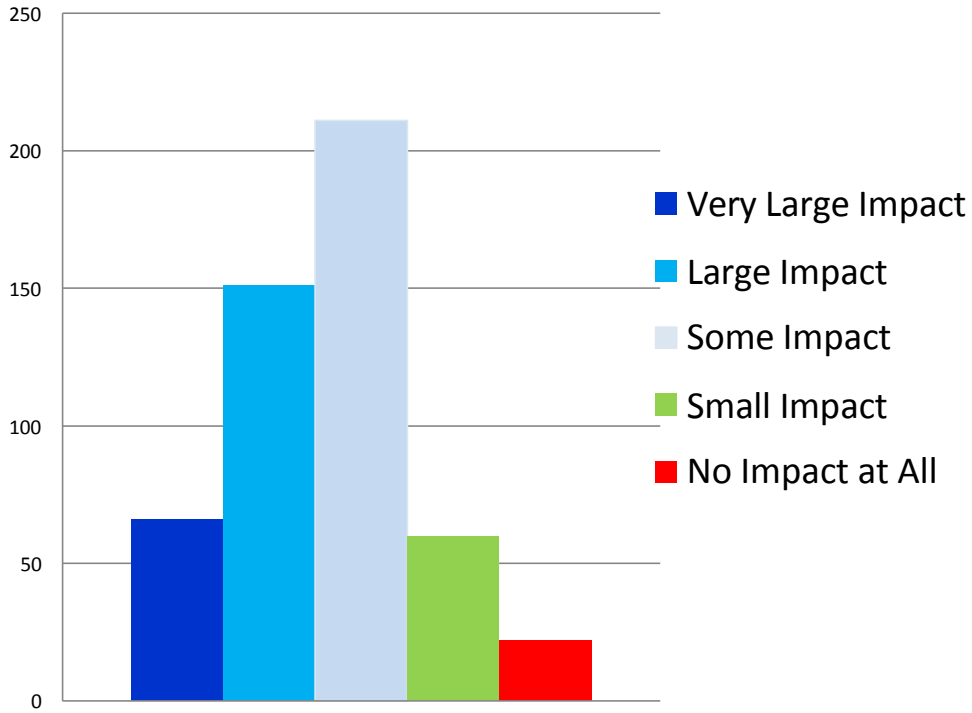
Survey Design

- Web-based survey created in “Survey Tracker”
- Survey consists of 14 questions
- Survey design: Cyril Gay and Sharon Drumm
- Database of answers stored on ARS web server
- Survey administered by Michael Witles
- Regulated process: Paperwork Reduction Act, OMB 83-I Form
- Survey submitted to 1000 persons via e-mail
- Data collected over a three month period
- Goal: 30-50% respondents

Results

- 510 respondents
- 128 producers and farmers
- 55,000 entries to assess in Excel

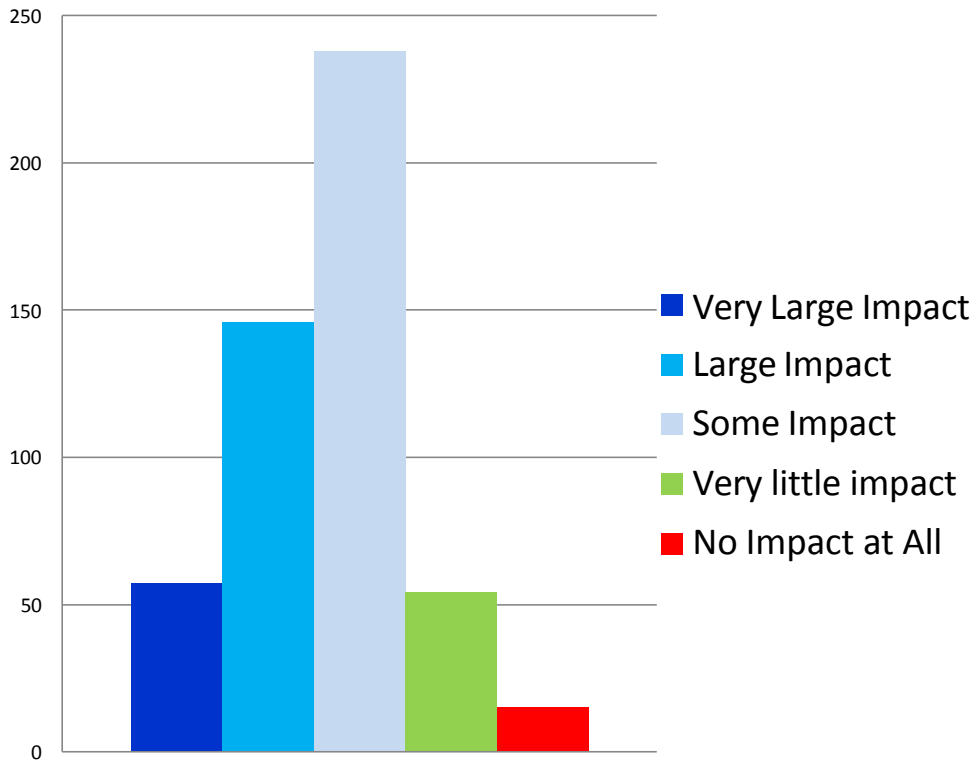
Is the Program having an Impact?



***Number of Respondents Total 510**

84% of respondents thought the program had at least some impact

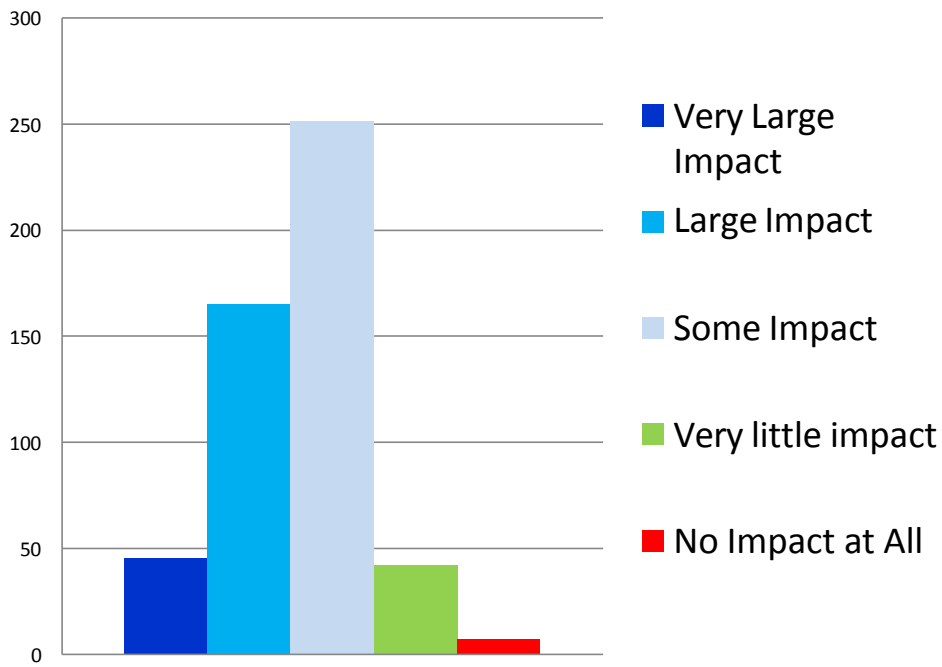
Impact of Program for Priority Diseases



***Number of Respondents Total 510**

86.5% of respondent thought the program had at least some impact

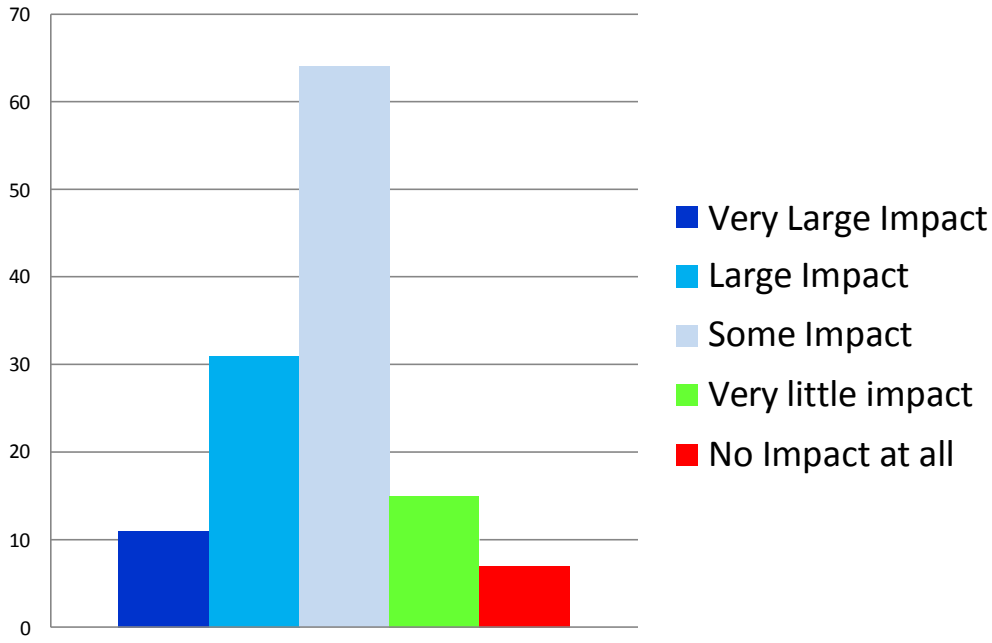
Program Impact Last 5-Years



***Number of Respondents Total 510**

90.4% of respondents thought the program had at least some impact

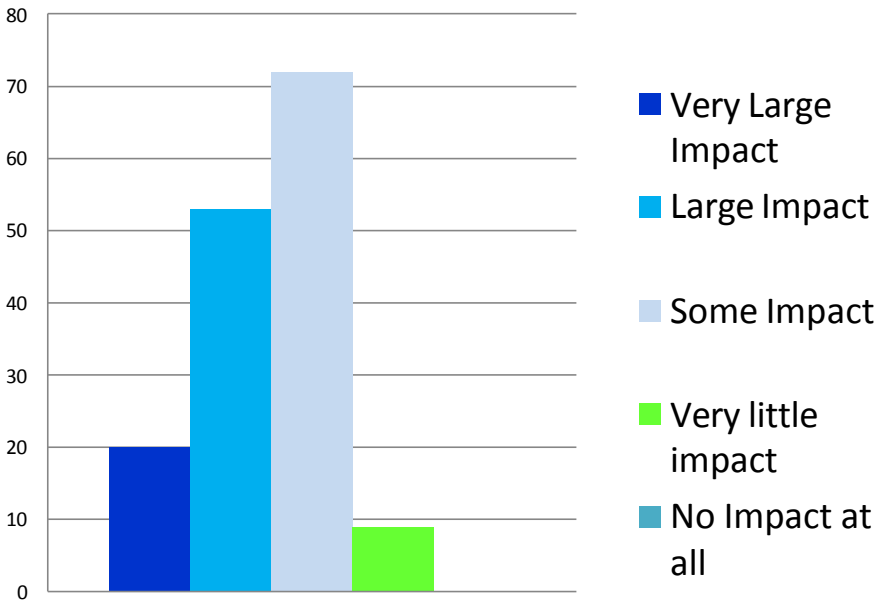
Producers and Farmers Program Impact Last 5-Years



***Number of Respondents Total 128**

83% of respondents thought the program had at least some impact

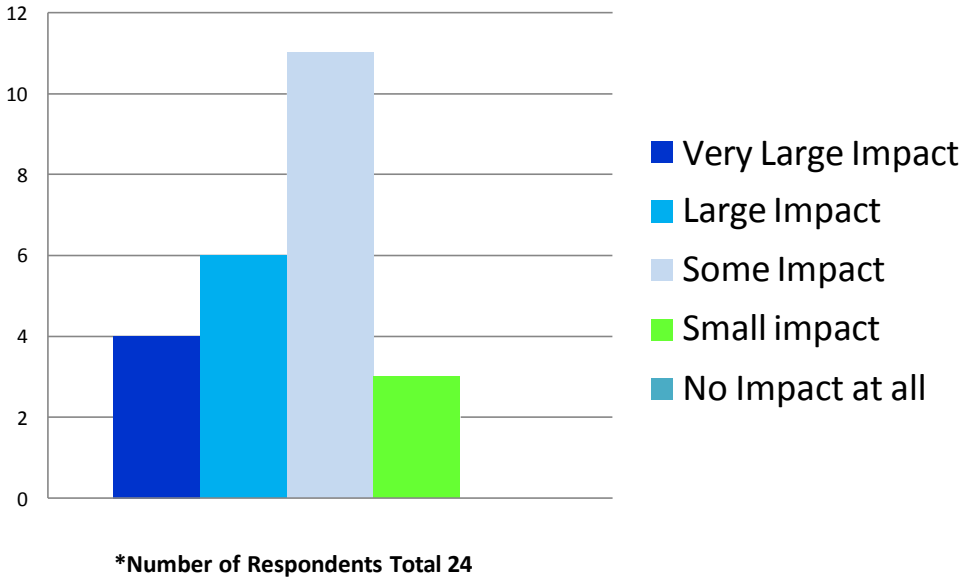
Universities Program Impact Last 5-Years



***Number of Respondents Total 154**

94% of respondents thought the program had at least some impact

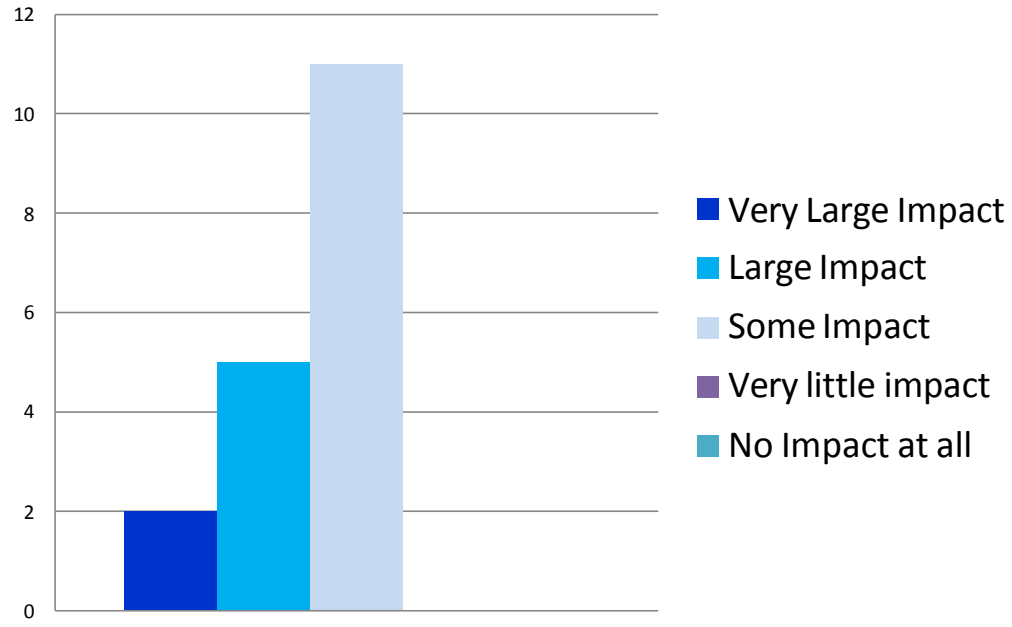
U.S. Government Agencies Program Impact Last 5-Years



***Number of Respondents Total 24**

87.5% of respondents thought the program had at least some impact

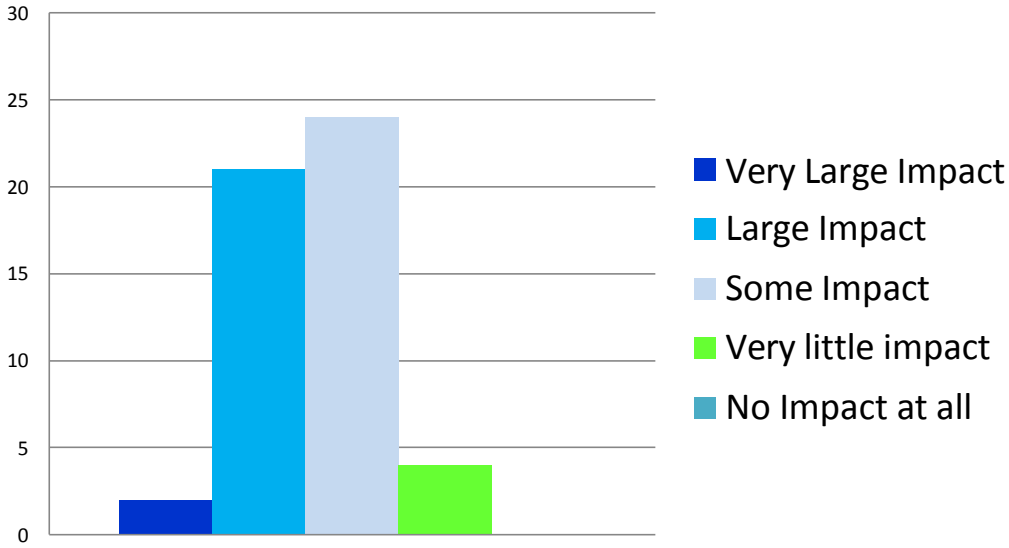
State Governments Program Impact Last 5-Years



***Number of Respondents Total 18**

100% of respondents thought the program had at least some impact

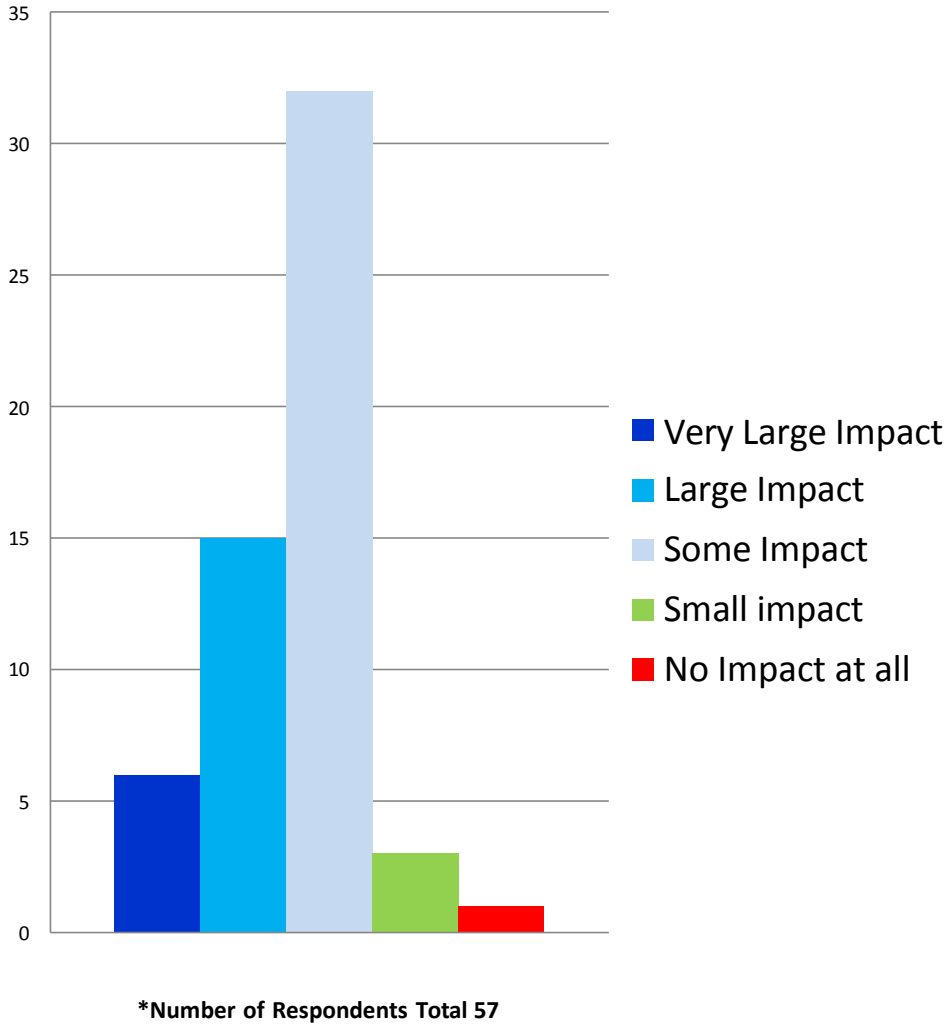
Pharmaceutical Companies Program Impact Last 5-Years



***Number of Respondents Total 51**

92% of respondents thought the program had at least some impact

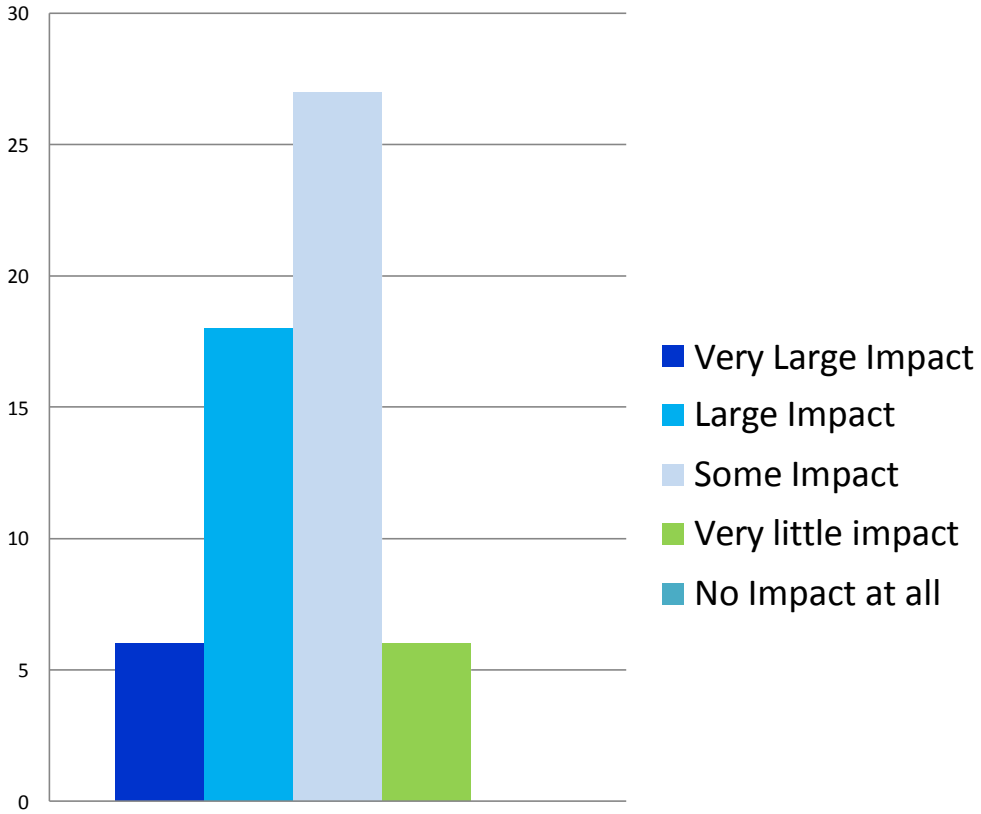
Program Impact: Beef Stakeholders



***Number of Respondents Total 57**

93% of respondents thought the program had at least some impact

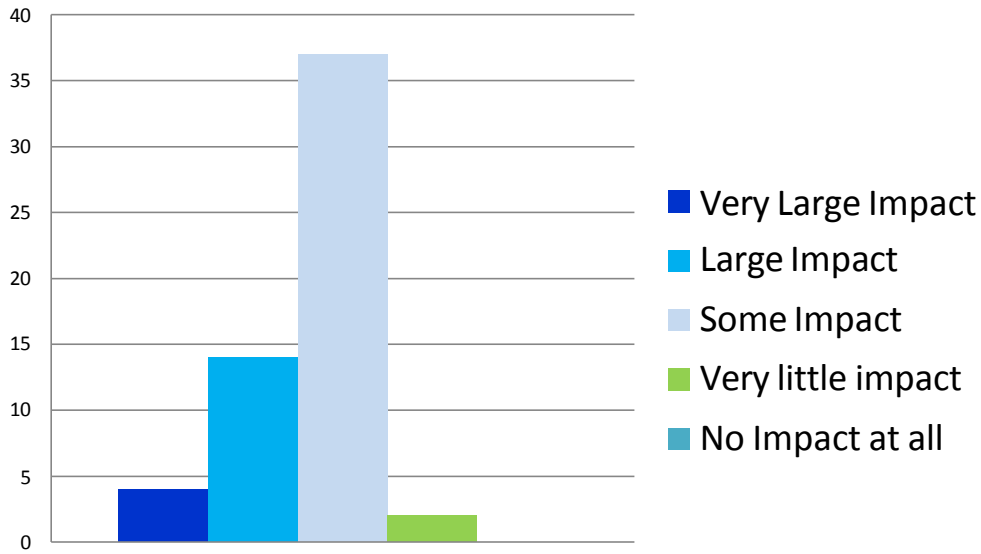
Beef Stakeholders Program Impact for Priority Diseases



***Number of Respondents Total 57**

89% of respondents thought the program had at least some impact

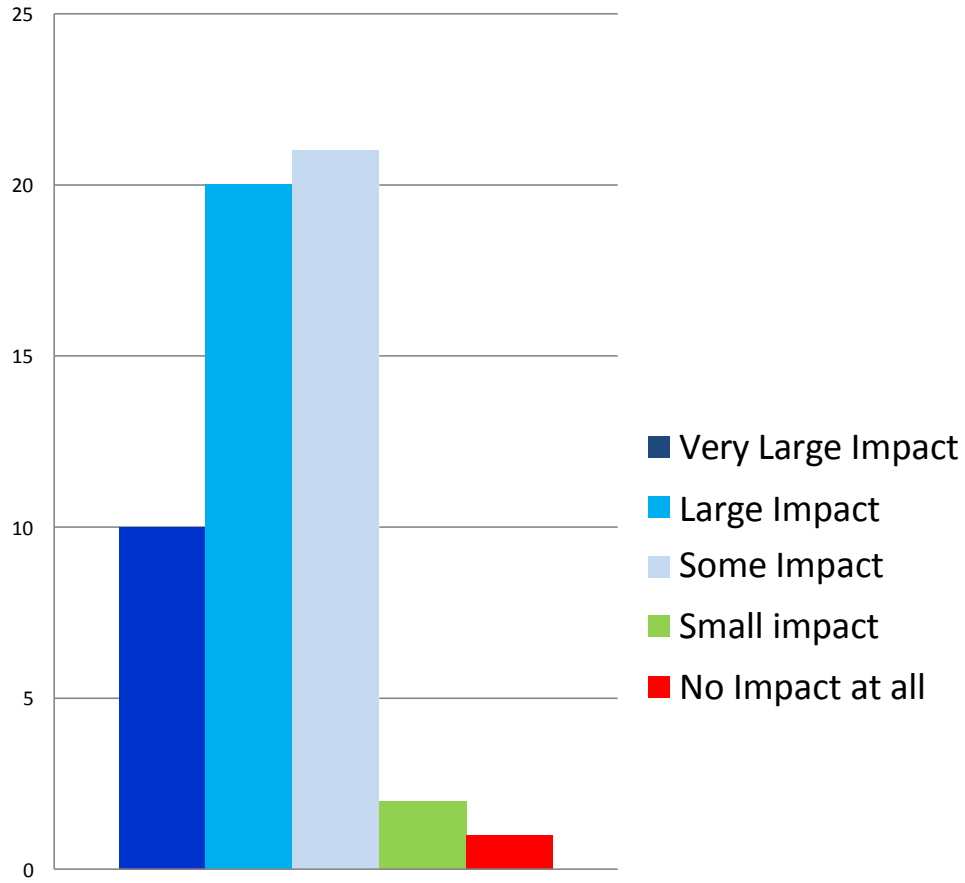
Beef Stakeholders Program Impact Last 5-Years



***Number of Respondents Total 57**

97% of respondents thought the program had at least some impact

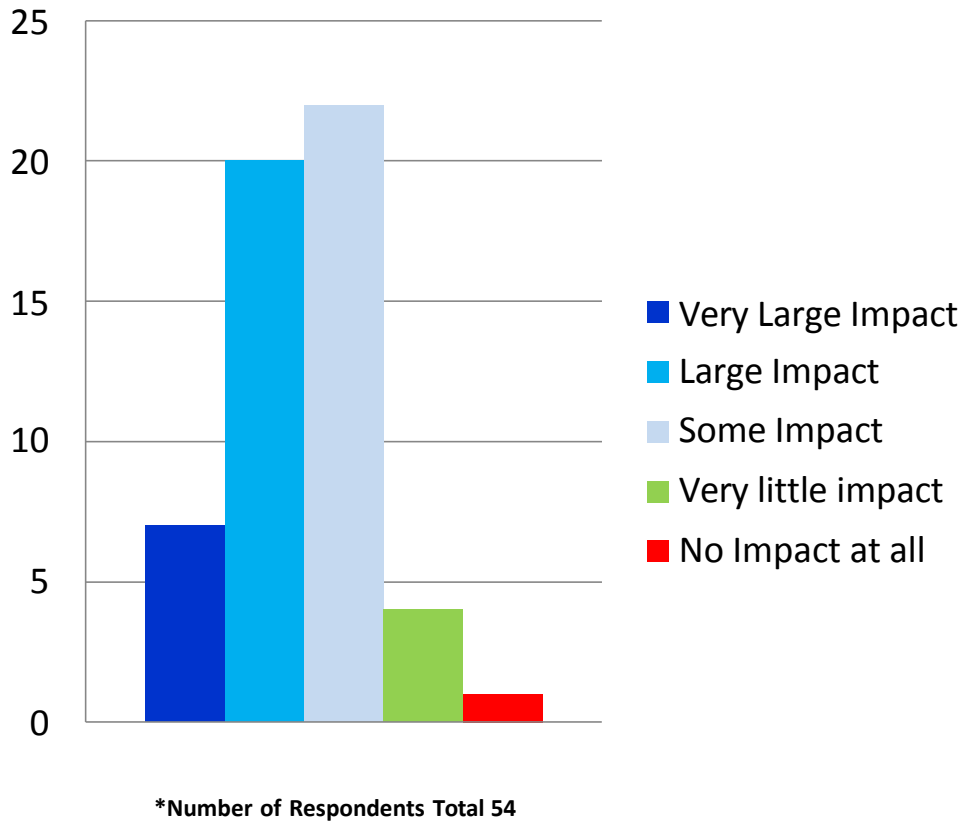
Program Impact: Poultry Stakeholders



***Number of Respondents Total 54**

94% of respondents thought the program had at least some impact

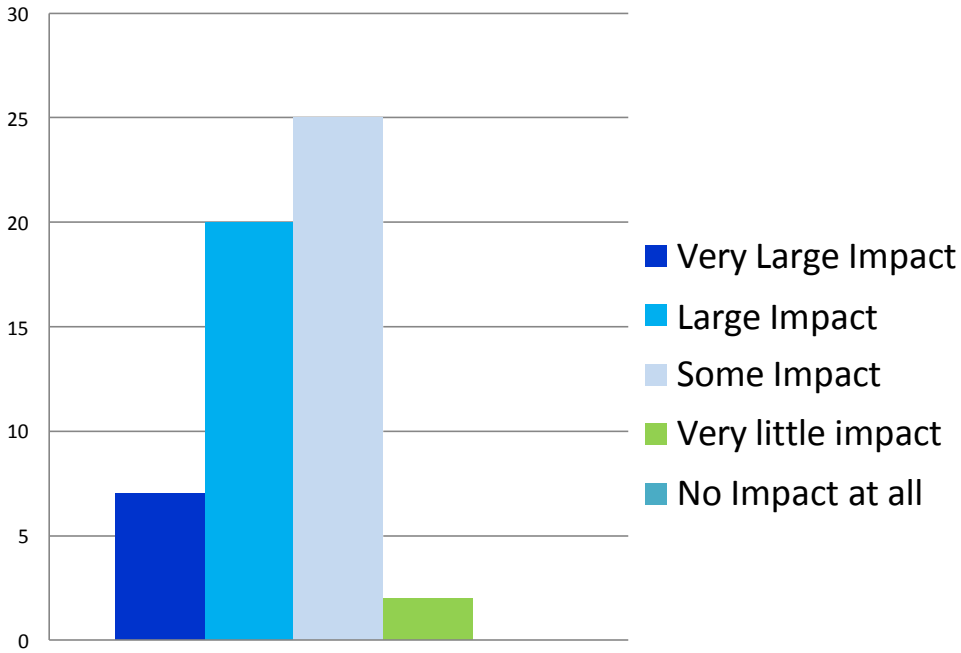
Poultry Stakeholders Program Impact for Priority Diseases



*Number of Respondents Total 54

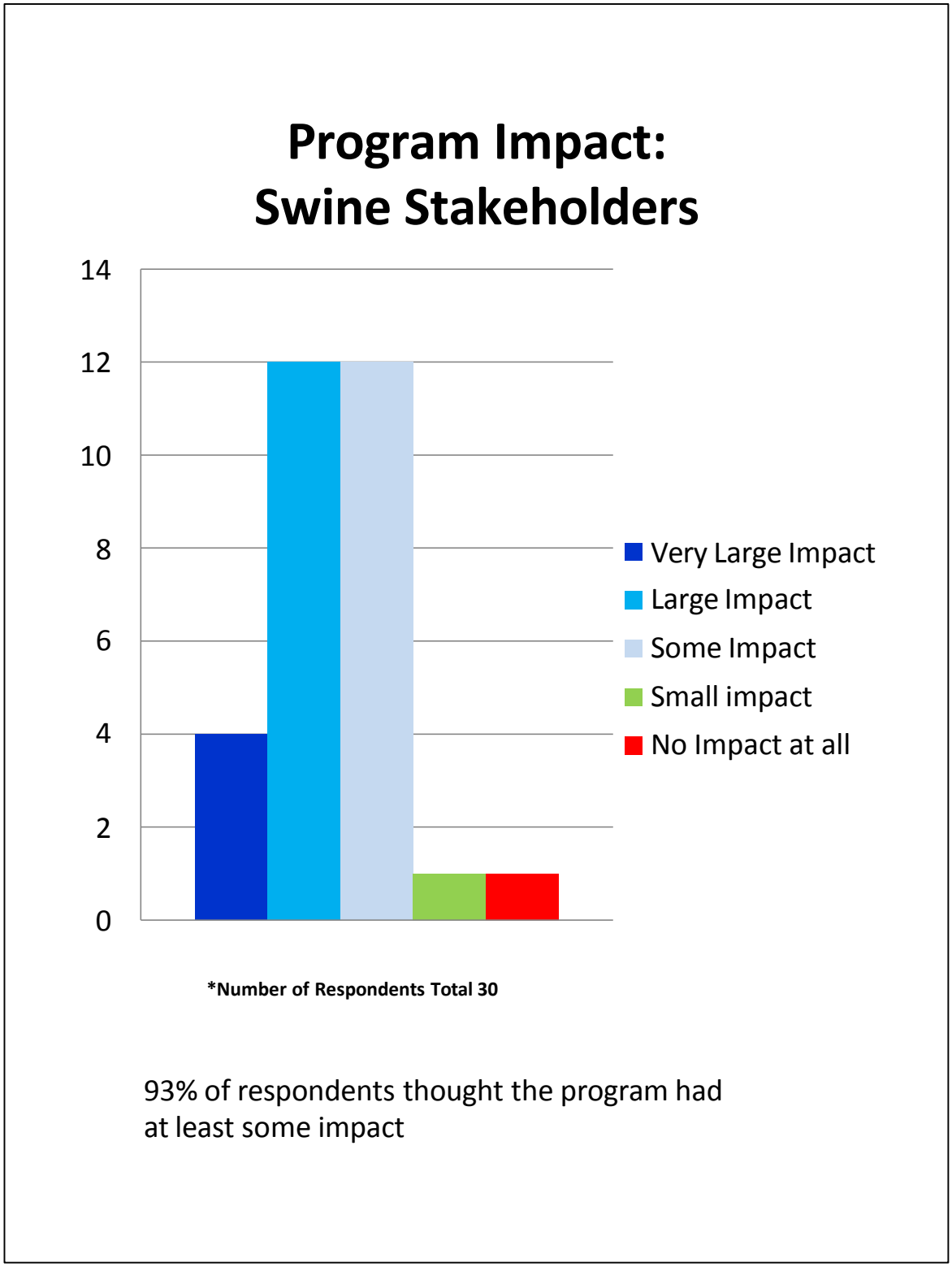
91% of respondents thought the program had at least some impact

Poultry Stakeholders Program Impact Last 5-Years

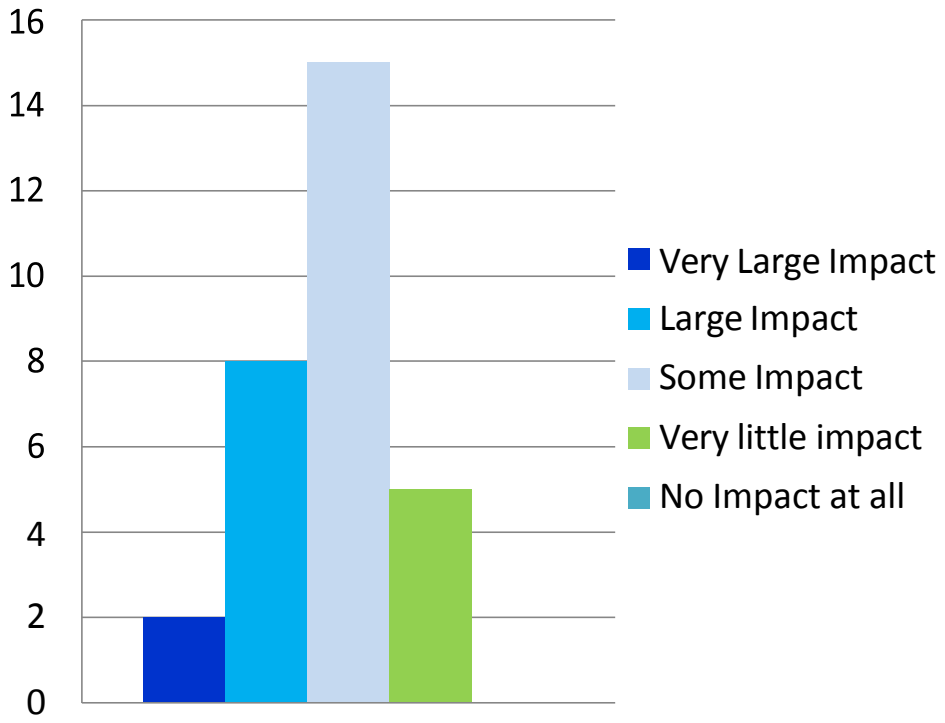


***Number of Respondents Total 54**

96% of respondents thought the program had at least some impact


Program Impact:
Swine Stakeholders
14
12
10
8
6
4
2
0
Very Large Impact
Large Impact
Some Impact
Small impact
No Impact at all
*Number of Respondents Total 30
93% of respondents thought the program had at least some impact

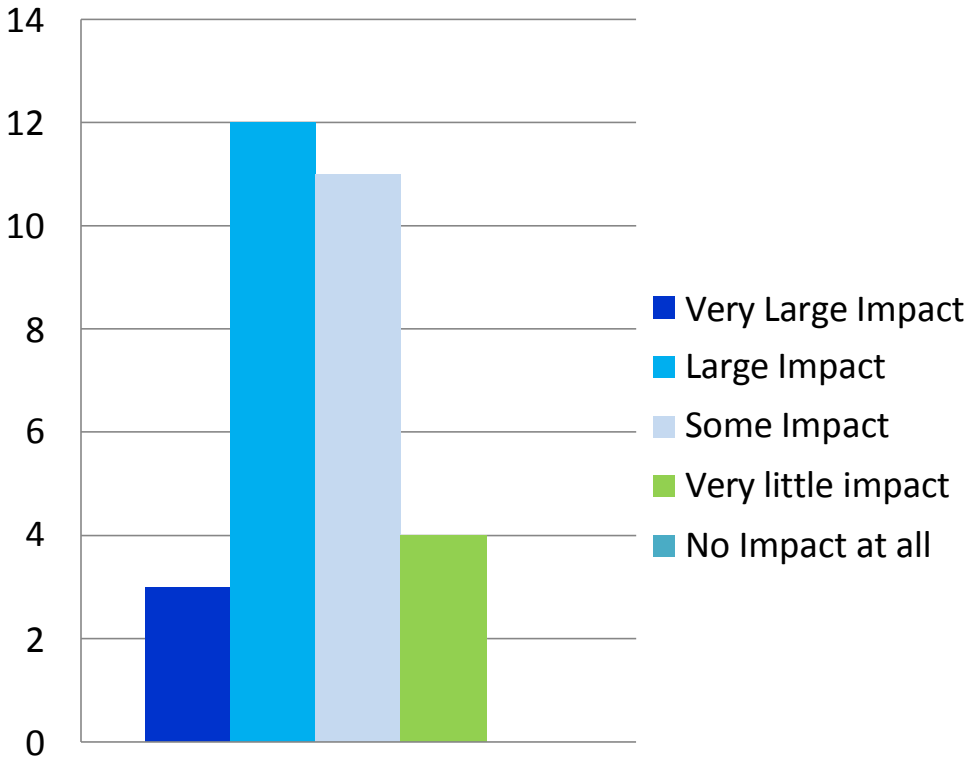
Swine Stakeholders Program Impact for Priority Diseases



***Number of Respondents Total 30**

83% of respondents thought the program had at least some impact

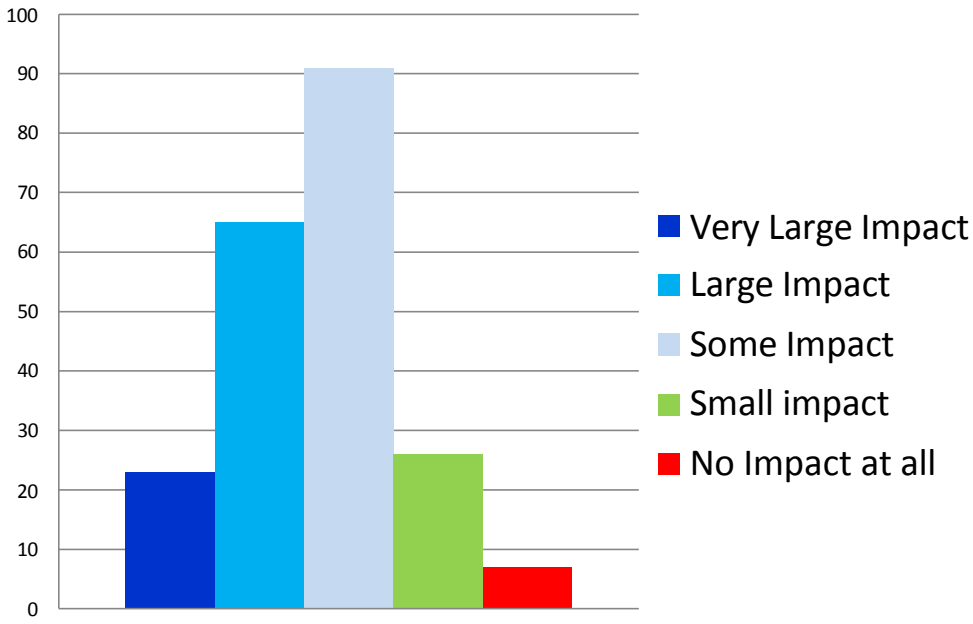
Swine Stakeholders Program Impact Last 5-Years



***Number of Respondents Total 30**

87% of respondents thought the program had at least some impact

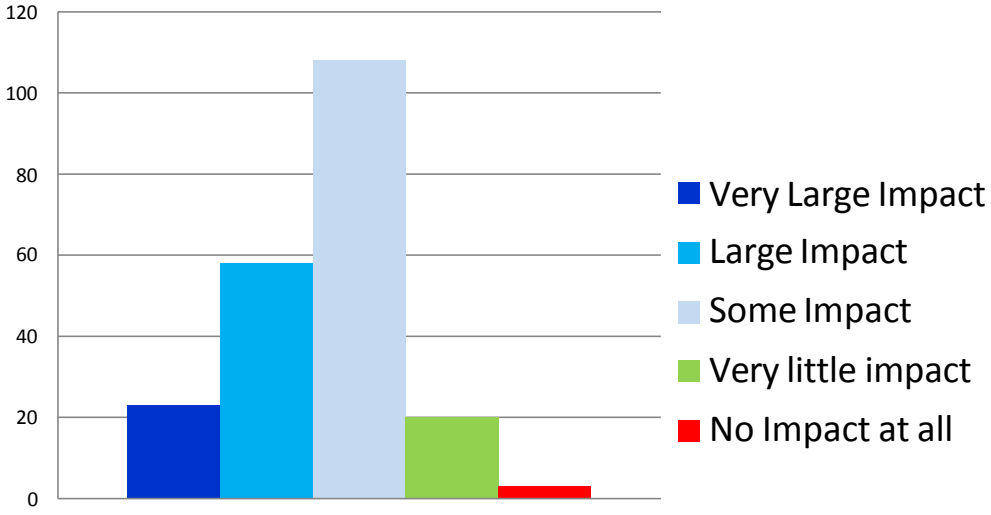
Program Impact: Dairy Stakeholders



***Number of Respondents Total 212**

84% of respondents thought the program had at least some impact

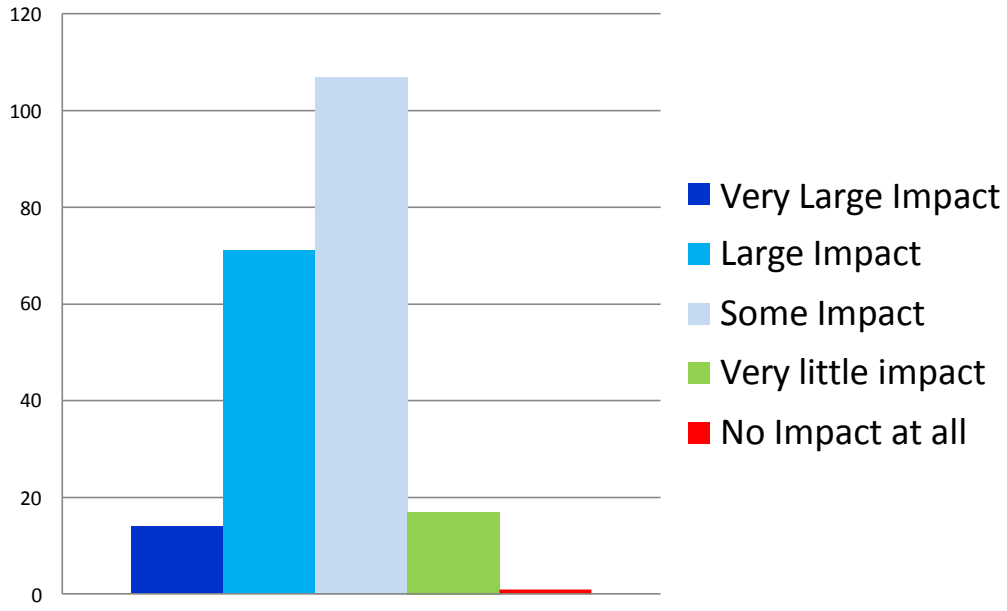
Dairy Stakeholders Program Impact for Priority Diseases



***Number of Respondents Total 212**

89% of respondents thought the program had at least some impact

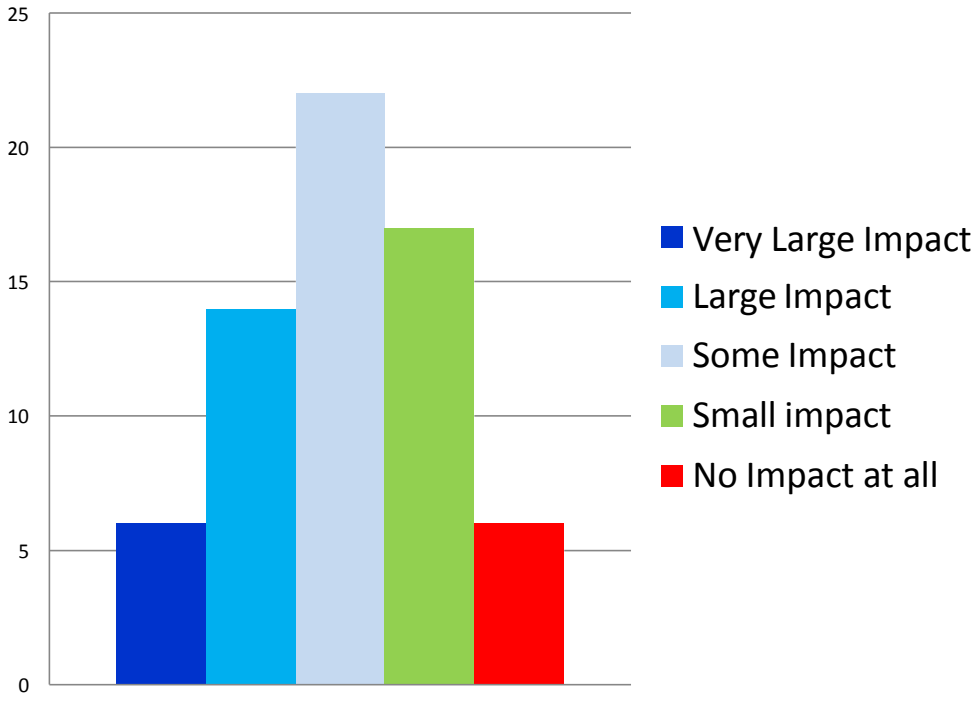
Dairy Stakeholders Program Impact Last 5-Years



***Number of Respondents Total 210**

91% of respondents thought the program had at least some impact

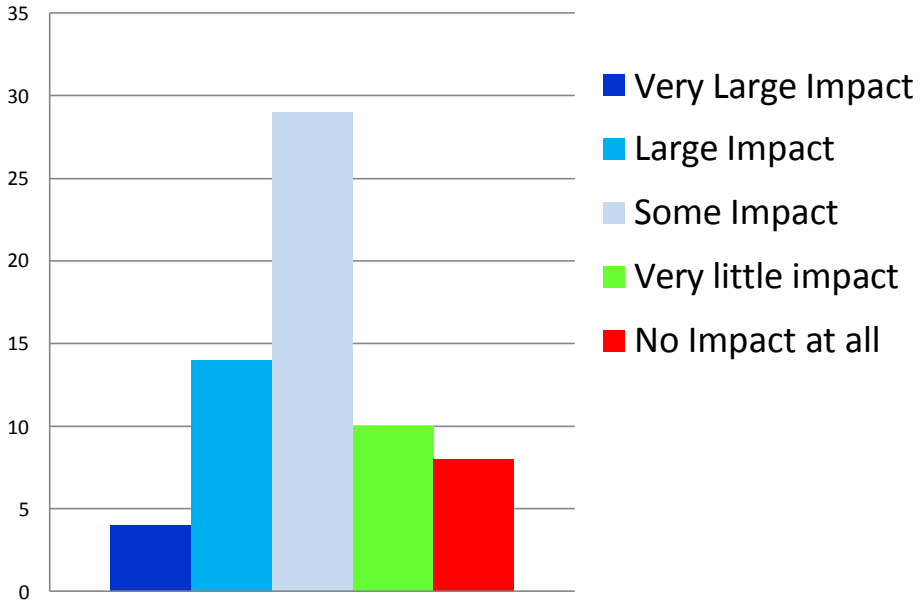
Program Impact: Sheep and Goats Stakeholders



***Number of Respondents Total 65**

64% of respondents thought the program had at least some impact

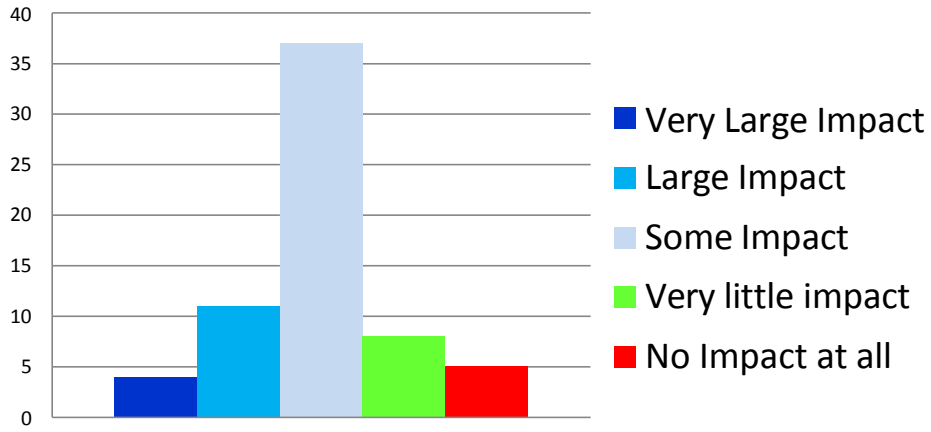
Sheep and Goats Stakeholders Program Impact for Priority Diseases



***Number of Respondents Total 65**

72% of respondents thought the program had at least some impact

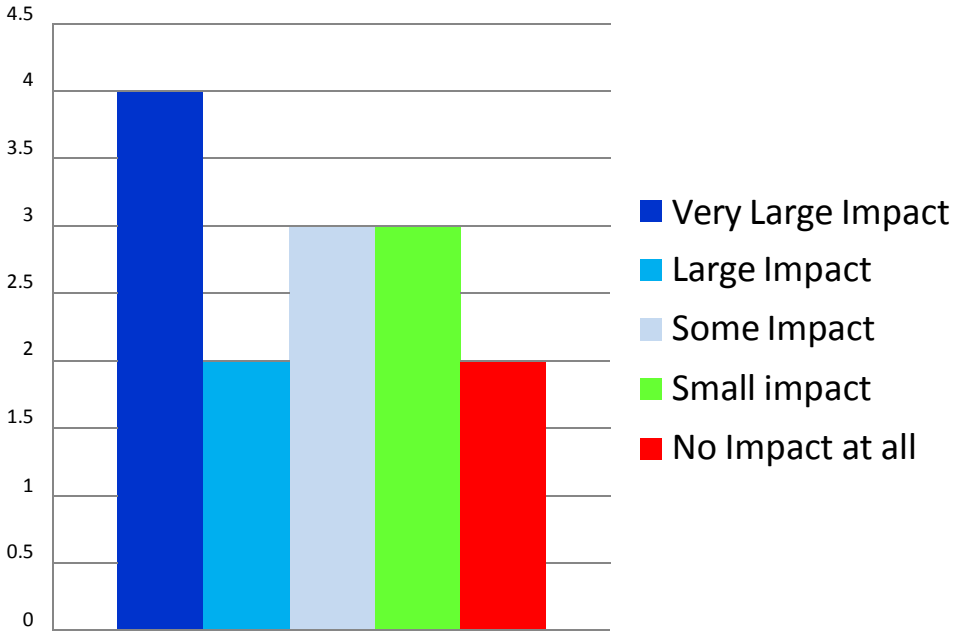
Sheep and Goats Stakeholders Program Impact Last 5-Years



***Number of Respondents Total 65**

80% of respondents thought the program had at least some impact

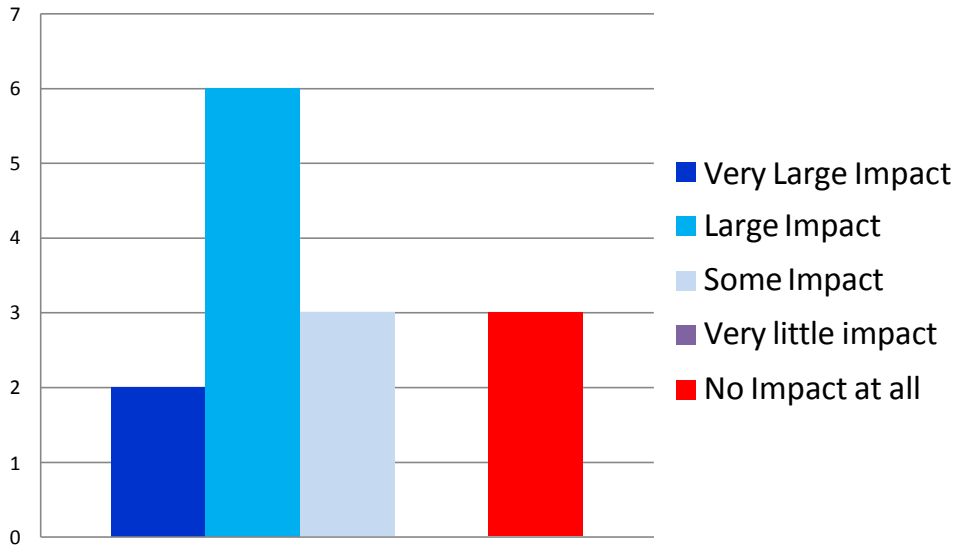
Program Impact
Wildlife and Captive Game Species



***Number of Respondents Total 14**

64% of respondents thought the program had at least some impact

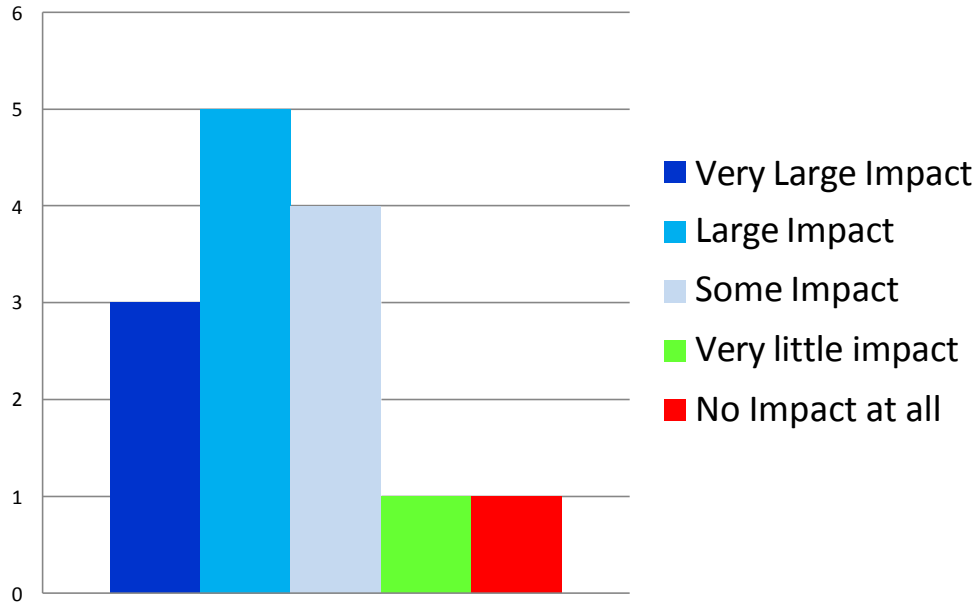
Wildlife and Captive Game Species
Program Impact for Priority Diseases



***Number of Respondents Total 14**

79% of respondents thought the program had at least some impact

Wildlife and Captive Game Species
Program Impact Last 5-Years



***Number of Respondents Total 14**

86% of respondents thought the program had at least some impact

Next Steps

- Complete data assessment
- Distribute report to the 1000 stakeholders and partners that were contacted
- Write National Program Assessment Report
- Hold national program assessment panel

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