

# **Action Plan**

## **National Program 103**

### **Animal Health**

#### **2012-2017**

#### **Goal:**

The goal of National Program 103, Animal Health, is to protect and ensure the safety of the Nation's agriculture and food supply through improved disease detection, prevention, control, and treatment. Basic and applied research approaches will be applied to solve animal health problems of high national priority. Emphasis will be given to methods and procedures to control animal diseases through the discovery and development of:

- Diagnostics
- Vaccines
- Biotherapeutics
- Animal genomics applications
- Disease management systems
- Animal disease models
- Farm Biosecurity measures

The vision for the program is to be recognized worldwide as a leader in animal health research that delivers effective solutions to prevent and control animal diseases that impact agriculture and public health.

The mission of the program is to deliver scientific information and tools to detect, control, and eradicate animal diseases of high national priority.

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 core competencies in bovine, swine, ovine and avian 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 expert collaborative research laboratories recognized by the World Organization for Animal Health, originally called and referred to as the Office International des Epizooties (OIE) Develop internationally recognized 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.

### **Background**

Investments in animal health research are critical to the growth and resilience of the supply of foods for tomorrow. Enhancing the health of animals in agricultural production systems will directly impact food quality and ensure a sufficient supply of macro and micro-nutrients to meet people's basic needs worldwide. When combined with other investments in agricultural development, research-based innovations will address some of the fundamental constraints that give rise to food insecurity by reducing production risks associated with pests and diseases.

Achieving results in animal health research in the 21<sup>st</sup> century will demand a "systems biology" approach in which knowledge obtained from animal genomes, functional genomics, clinical trials, and epidemiology are integrated in the discovery and development of countermeasures for preventing and controlling disease outbreaks.

Accordingly, this national program will foster the alignment of research expertise and the establishment of strategic partnerships to maximize productivity and impact. For this purpose, NP 103 projects will be aligned under eight research components. Each component includes problem statements that define the scope of the action plan. Research components draw upon relevant expertise within NP 103, coordinating and integrating that expertise to develop a specific useful application of the knowledge. Projects within the research components should also attract additional federal, university, and industry partners at both the national and international level. The aim of these partnerships will be to support component projects. Their inclusion will enable and enhance, rather than detract from, the anticipated products of the component projects. Because a significant number of projects in the animal health research portfolio focus on the discovery of novel technologies, intellectual property strategies will be addressed in the project plans developed by ARS scientists to facilitate technology transfers and help foster investments by the private sector in the development of these technologies.

The anticipated products of the animal health program will aim for the following targets:

- Enhance "Global Food Security" by finding solutions to problems incurred by domestic and foreign animal diseases of livestock and poultry.
- Support "One Health" initiatives by implementing research programs that will benefit the animal health, public health, and the biomedical research communities.
- Advance the productivity frontier by supporting "Feed the Future" initiatives.

- Develop methods to help producers adjust to changing farming practices that will allow consumer driven issues to be accommodated without compromising financial viability.
- Establish methods to detect, analyze, and respond to new and emerging pathogens that threaten agriculture and public health.
- Find solutions to create and maintain a barrier to pathogens at the domestic-wildlife interface.
- Build integrated research programs to discover genetic variations associated with disease susceptibility and resistance to increase our farmers' productivity and competitiveness.
- Develop 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.

**Relationship of This National Program to the USDA/ARS Strategic Plans:** This Action Plan addresses the high level goals and objectives of the USDA 2010-2015 Strategic Plan, Objectives 3.3 (Support Sustainable Agriculture Production in Food – Insecure Nations) and 4.4 (Protect Agricultural Health by Minimizing Major Diseases and Pests to Ensure Access to Safe, Plentiful, and Nutritious Food), and the ARS 2006-2011 Strategic Plan, Strategic Goal 4 (Enhance Protection and Safety of the Nation's Agriculture and Food Supply), Objective 4.2 (Reduce the number, severity and distribution of agricultural pest and disease outbreaks). Two performance measures set the targets for NP 103 research within the USDA ARS 2006-2011 Strategic Plan:

**Performance Measure 4.2.1:** Provide scientific information to protect animals, humans, and property from the negative effects of pests, infectious diseases, and other disease-causing entities. **Target:** Cumulatively, complete 10 research studies that have significant impact on the scientific community, leading to development of technologies for the integration of prevention and treatment strategies to manage top priority endemic and exotic threats to livestock, humans, and property.

**Performance Measure 4.2.2:** 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. **Target:** Cumulatively, transfer five technologies to the commercial and government sectors.

**Research Component Overview:**

The NP 103 Action Plan contains general strategies and specific actions within the following organizational hierarchy: 1) Components, which are general categories of agriculturally useful research areas identified with the help of stakeholders; 2) Problem Statements, indicating the specific nature and scope of problems to be solved by ARS; and 3) Research Needs, which are the kinds of research to be performed by ARS in order to achieve a successful resolution of the problem. The components of the program are:

### Component 1: Biodefense

Problem Statement 1A: Foreign Animal Diseases

Problem Statement 1B: Emerging Diseases

### Component 2: Animal Genomics

Problem Statement 2A: Oncogenic Diseases of Poultry

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

Problem Statement 2C: Bovine Mastitis

Problem Statement 2D: Increasing Gut Health and Disease resistance

Problem Statement 2E: Genetics of Prion Disease Susceptibility

### Component 3: Zoonotic Diseases

Problem Statement 3A: Brucellosis

Problem Statement 3B: Leptospirosis

Problem Statement 3C: Tuberculosis

### Component 4: Respiratory Diseases

Problem Statement 4A: Bovine Respiratory Diseases

Problem Statement 4B: Porcine Respiratory Diseases

Problem Statement 4C: Poultry Respiratory Diseases

### Component 5: Enteric Diseases

Problem Statement 5A: Johne's Disease

Problem Statement 5B: Enteric Diseases of Poultry

### Component 6: Parasitic Diseases

Problem Statement 6A: Gastrointestinal (GI) Parasitic Diseases

Problem Statement 6B: Hemoparasitic Diseases

### Component 7: Transmissible Spongiform Encephalopathies

Problem Statement 7A: Nature and Origin of Prion Agents

Problem Statement 7B: Pathobiology of Prion Strains

Problem Statement 7C: Determinants of Transmissibility and Epidemiology

Problem Statement 7D: Diagnostics, Detection, and Surveillance

### **Component 1: Biodefense**

The ARS mission is extensively linked to the President's National Strategy for Countering Biological Threats. ARS has unique and critical resources dedicated to ensuring that agricultural production is secure, sustainable, and efficient with the aim of providing American consumers with a healthy, safe, and affordable food supply. Many of these responsibilities involve protecting people, crops, livestock, poultry, and other living resources from pests and pathogens with the potential to cause severe economic consequences and/or public health incidents.

Because many dangerous pathogens have the potential to rapidly spread across national borders, ARS maintains a global view of the biological threats to food and agriculture. Animal production is always threatened by diseases, naturally or deliberately introduced, into a naïve healthy population of productive animals. These diseases vary in the degree of economic loss they cause, their potential to spread, and ease of control and eradication. Furthermore, each year new disease-causing agents are discovered, known organisms mutate to previously unrecognized forms, and new pathways of agent introduction are created. Therefore, in the face of uncertainty and the inability to protect against every conceivable microbiological attack, the best biodefense program for countering biological threats of livestock and poultry is one which increases Biosecurity on farms, provides tools for increased disease surveillance, increases innate animal defenses, provides tools that treat diseased animals easily, and rapidly allows farmers to return to production as soon as possible.

Since many of the worst animal pathogens do not exist in the United States, disease research must extend to countries where the diseases exist. Partnerships with research organizations in other countries are therefore essential in implementing a biodefense research program against animal disease outbreaks. The program must include research on how a disease agent survives outside of the host, how the organism moves between susceptible hosts, how the pathogen attacks the animal, and how it then escapes from the host. Increased research on how pathogens move between countries and between farms will allow prevention programs to enhance on-farm Biosecurity and reduce the chance of pathogen introductions. In order to respond to a disease incursion, research must provide tools for accurate and continuous surveillance and vaccination programs. To counter an animal disease, research programs must also consider ways to manipulate the animal's immunological resistance to infection and ways to increase disease resistance through genetic selection, which may be the best way to minimize economic loss and disease spread. To ensure producers are able to return to full production and export their products as soon as possible, research must also provide the means to prove that animals are free of the disease.

#### **Problem Statement 1A: Foreign Animal Diseases**

Foreign animal diseases represent a major threat to U.S. agriculture. At the March 2010 Animal Health Planning Workshop, stakeholders indicated that biodefense research on foreign animal diseases is a top priority—ranked 5<sup>th</sup> by the beef industry, 3<sup>rd</sup> by the poultry broiler industry, and 6<sup>th</sup> by the swine industry. Introduction of these agents, either accidental or deliberate, has devastating social and economic effects not only in the country's agricultural systems but also in a wide range of economic activities. Diseases in this component include but are not limited to Foot-and-Mouth Disease, Peste des Petits Ruminants, Avian Influenza, virulent Newcastle disease, Rift Valley Fever, Classical Swine Fever, African Swine Fever, Vesicular Stomatitis, Exotic bluetongue, and Lumpy skin disease. Additional diseases that impact trade are addressed under Component 7, Hemoparasitic Diseases, and Component 8, Bovine Spongiform Encephalopathy (BSE).

Animal health officials define an exotic or foreign animal disease (FAD) as an important transmissible livestock or poultry disease believed to be absent from the United States

and its territories that has the potential to be a significant health or economic impact if it enters the country. Foreign animal diseases are considered a threat to the United States when they significantly affect human health or animal production and trade, or when there is an appreciable cost associated with disease control and eradication efforts. To protect the long-term health and profitability of U.S. animal agriculture, incursions of an FAD must be prevented or rapidly controlled.

In the United States, control usually means disease eradication. Disease eradication is currently accomplished by eliminating the animal, resulting in loss of protein, loss of income to the farm community, public opposition, and environmental disruption. In addition to control costs, one of the most immediate and severe consequences of a FAD occurrence in the United States is the loss of export markets. As we move into the 21st century, many new issues and factors are affecting FAD prevention, control, management, and recovery. These factors include free trade agreements, free trade blocks, regionalization, increased international passenger travel, intensification of animal production, the constant evolution of infectious agents, and the uncertain impact of biotechnology and bioterrorism.

Current methods for prevention and control of high consequence diseases, including prevention, detection, control, and eradication, are not socially or economically acceptable. Rapid detection and characterization tools for prevention control and eradication of foreign animal diseases are inadequate or not currently available. Further, our understanding of pathogenesis, transmission, and immune response is insufficient to rapidly control and eradicate foreign animal diseases, while effective measures to prevent, control, and eradicate foreign animal diseases are lacking or inadequate.

#### **Research Needs:**

In order to control foreign animal disease, a wide variety of agent detection platforms need to be developed and validated. Information for design of these platforms will come in part from further knowledge of pathogen genomics and proteomics and in part from understanding the evolution and genetic variability of disease agents. Although many of the foreign animal diseases have existed for many years in many countries, there is still much more fundamental knowledge of these agents that is required. There is still a lack of understanding in host range and tissue tropism, carrier state, duration and routes of shedding, transmission mechanisms (e.g. vectors, fomites, aerosols), ecology, and epidemiology (e.g., wildlife reservoirs). If these diseases should occur in the United States, more effective prevention and control tools—such as suitable control strategies compatible with short time and cost of recovery from disease outbreaks—need to be developed. There is also a need for vaccines that can differentiate infected and vaccinated animals (DIVA) and biotherapeutics suitable for strategic stockpiles, as well as integrated methods of disease control—including vector control and animal management; all of these tools lead to a better capability to regain country disease-free status and retain economic sustainability.

**Anticipated Products:**

- New solutions to prevent economic losses from foreign animal diseases in agricultural and wild-life species.
- Effective countermeasures to prevent and eliminate the threat of zoonotic diseases in agricultural and wild-life species.
- Scientific information to establish on-farm practices that will maximize “Biosecurity” to protect farms from naturally or intentionally introduced pathogens that threaten food security, farm productivity, and the trade and export of agricultural products.
- Experimental animal disease models that will serve the veterinary and public health research communities to significantly shorten the timelines for developing breakthrough medicines and disease prevention tools.
- Integrated predictive modeling capability for emerging and/or intentionally released infectious diseases of animals and the collection of data to support these models.
- Novel detection systems and broad spectrum vaccines and biotherapeutics to counter the threat of emerging diseases or engineered biological weapons.
- Novel countermeasures against the natural or intentional introduction of agricultural threats, including new methods for detection, prevention, and characterization of high-consequence agents.

**Potential Benefits:**

Successful completion of this research will result in scientific information necessary to advance the discovery of countermeasures to prevent, control, eradicate, and recover from foreign animal disease outbreaks. Foreign animal diseases pose a tremendous threat to people and our public health systems. Preventing and controlling foreign animal diseases at the source are the most efficient and cost-effective means of protecting farmers and people. In a world where trade is a fundamental component of everyday life, having scientific information to develop countermeasures for rapid control and eradication of diseases that limit or prevent animal and animal product trade is extremely relevant to livestock and poultry producers, animal health professionals, and federal agencies responsible for emergency management and preparedness. The ARS foreign animal disease research program will benefit a large component of our society, as outbreaks of foreign animal diseases not only affect the agriculture sector, but also widely reach most sectors of the economy.

**Problem Statement 1B: Emerging Diseases**

Several new emerging animal disease issues appear every year. Many factors—globalization of trade, movement of masses of people and agricultural products, changing weather patterns, rapid population growth in cities, intensive agriculture, limited genetic diversity in farm animals, changes in farm practices—are creating new opportunities for the re-emergence and spread of infectious diseases, including those resistant to antibiotics in both humans and livestock. Exotic (non-native) organisms, once introduced into the United States, can escalate into an epidemic because of the absence of vaccines or effective drugs, lack of resistance in host animals, and limited resources to effectively

manage the spread of such pathogens. A coordinated national collaborative research program integrating ARS core competencies in infectious diseases, virology, bacteriology, diseases complexes, microbial genomics, pathology, disease detection, and epidemiology is needed to identify new pathogens and predictors of emerging diseases of livestock.

At the March 2010 Animal Health Planning Workshop, stakeholders indicated that emerging diseases is the highest priority of the equine industry, the 2<sup>nd</sup> priority of the swine industry, and the 3<sup>rd</sup> priority of the beef industry. According to the U.S. swine industry, for instance, the emergence of the novel H1N1 virus cost industry \$1.3 billion. In demonstrating that the virus was not in pig meat, ARS' rapid response was critical to regaining consumer confidence in pork as well as opening doors shut by trading partners.

**Research Needs:**

The capability to rapidly identify, characterize, control, and eradicate new animal pathogens of high economic consequence is not well developed. Specifically, there is a need to isolate, identify, and characterize pathogens associated with new disease complexes of unknown etiologies. Scientists need to conduct challenge studies to fulfill Koch's postulates and determine the pathogenesis of monovalent and multivalent infections. Once a new agent has been identified and isolated, there is a need to sequence partial or complete microbial genomes to identify unique sequences for diagnostic discovery and molecular epidemiology research. Research to identify mechanisms of disease, disease transmission, and host range specificity to determine the prevalence and emerging potential of new diseases is also needed. All of these procedures need to be done in real time to minimize the impact to the industry involved. Ultimately, good research will lead to predictors of disease emergence and disease outbreaks and the development of the appropriate intervention strategies, which may include new vaccine candidates, antimicrobial therapies, or eradication strategies.

**Anticipated Products:**

- New pathogens associated with emerging diseases identified.
- Predictors of emerging livestock diseases.
- Methods to rapidly detect and characterize the etiology of new and emerging diseases.
- Tools and expertise to prevent emerging diseases and rapidly implement intervention strategies to respond to new disease outbreaks.

**Potential Benefits:**

This research will yield information about transmission and pathogenesis and intervention strategies to enable detection, control, and eradication of new emergent diseases.



### **Component 1 Resources:**

The following ARS locations have research projects addressing the problem statements identified under Component 1:

- Ames, IA
- Athens, GA
- Manhattan, KS
- Orient Point, NY

### **Component 2: Animal Genomics**

Animal genomics is a new field of research defined by rapidly evolving technologies derived from the recent breakthroughs in sequencing whole animal genomes. Although the field originated decades ago with the identification of genetic markers, which led to the assembly of genetic and physical maps, the promise of animal genomics could not be realized until the sequencing and annotation of the human genome was achieved in 2002. The human genome project has since generated ground-breaking research tools such as the Haplotype map of the human genome, consisting of the genome-wide detection and characterization of single nucleotide polymorphisms (SNPs). These genome-enabled technologies have galvanized the biotechnology industry and are fostering the development of powerful tools in biomedical research. Importantly, these initial accomplishments have highlighted the need for sequencing animal species and the need for comparisons to fully explore the unknown world of genomic information.

The animal health research community is now in a position to capitalize on the infrastructure built by the human genome project with the sequencing of important animal species such as the chicken (*Gallus gallus*), the cow (*Bos taurus*), the pig (*Sus scrofa*), and the horse (*Equus caballus*) genomes. Together, these projects provide the foundation for the field of animal genomics. A critical area will be the sequencing of genomes within animal species that demonstrate differences in disease susceptibility. Initial results of whole genome comparisons are already predicting that the field of comparative genomics will yield rich returns in biomedical research. For example, animal models have already been successfully used to address human diseases and new tools developed to create comparative phenotypic models. Likewise, the animal health research community now has access to powerful research tools to discover new effective measures to prevent, diagnose, and treat diseases.

To capitalize on the tremendous opportunities these new tools offer for animal health, animal genomics research is organized under Component 2 to align projects where genomics is likely to have the biggest impact: Oncogenic Diseases of Poultry; Highly Infectious Diseases of Livestock and Poultry; Bovine Mastitis; and Improving Gut Health and Disease Resistance. The aim of the research is solve problems within the following three priority areas: 1) Marker-assisted selection of animals with desirable health traits; 2) Functional genomics of host-pathogen interactions; 3) Translating genomics information into tools for controlling diseases. Two international symposia, Animal Genomics for Animal Health (AGAH)--organized by ARS in 2007 and 2010 in

collaboration with the French National Institute for Agricultural Research (INRA) and the World Organization for Animal Health (OIE)--provided key recommendations for solving problems within these three priority areas, and form the basis for the potential outputs of this research component.

The possible impact and importance of investing in animal genomics research was highlighted at our March 2010 Animal Health Planning Workshop. Stakeholders from the poultry industry identified functional genomics to understand disease resistance as their 1<sup>st</sup> priority. The equine industry identified genomics research as their 4<sup>th</sup> priority. Likewise, the swine industry identified genomics and genetic resistance in controlling porcine reproductive and respiratory syndrome (PRRSV) as one of their top research priorities, and metagenomics research on the microbiome for healthy pig production as their 5<sup>th</sup> priority. The sheep industry identified the immunogenetics of ovine progressive pneumonia (OPP) as their 3<sup>rd</sup> priority, and genomics solutions to economically significant diseases of sheep their 5<sup>th</sup> priority. Stakeholders representing specialty farm animals identified comparative genomics to define species differences in susceptibility to bacterial pneumonia as their 4<sup>th</sup> priority. The turkey industry identified metagenomics research on the microbiome to improve gut health as their 4<sup>th</sup> priority.

#### **Problem Statement 2A: Oncogenic Diseases of Poultry**

Oncogenic viruses of poultry are endemic in the United States and cause periodic outbreaks with severe economic loss. The continued circulation of these viruses in commercial flocks lead to shifts in viral virulence or the emergence of new subgroups through mutation and/or recombination. Control measures consist of blanket vaccination of all commercial birds, coupled with diagnostic testing procedures to ensure breeder flocks remain virus free. These control measures cost the U.S. poultry industry in excess of \$200 million for vaccination and \$20 million in diagnostic tests annually—a conservative estimate. More importantly, these viruses have been evolving for more than 50 years, continually rendering the latest control measures ineffective.

Oncogenic viruses are associated with three economically important neoplastic diseases of poultry, namely Marek's disease (MD), caused by a herpesvirus, and avian leukosis and reticuloendotheliosis, caused by retroviruses. Avian leukosis virus (ALV) and reticuloendotheliosis virus (REV) are the most common naturally occurring retroviruses associated with neoplastic diseases in poultry. In addition to causing tumors and other production problems, both ALV and REV are potential contaminants of live-virus vaccines of poultry. In 2004, for instance, a new recombinant virus, consisting partly of ALV-A and partly of ALV-E, was isolated from contaminated Marek's disease vaccine.

Control of retroviruses in poultry is complicated by lack of specific diagnostic reagents and vaccines. This lack of specific reagents, coupled with the high rate of retrovirus mutations and recombination, produces a commercial environment capable of generating a high frequency of antigenic and molecular variations among strains of the virus. During the 1990s, this environment produced an emerging ALV capable of inducing myeloid leukosis termed, ALV-J. The generation of ALV-J through recombination threatened the economic viability of the entire broiler industry and immediately became the industry's

highest disease priority. As a result of this outbreak, poultry breeders routinely test their breeder flocks for the presence of ALV. However, the absence of strategies that will enable the identification and control of new recombinant strains of ALV can result in devastating economic losses in meat- and egg-type breeder flocks. In addition to being a possible contaminant of biologic products, REV infection can create a barrier to exporting breeding stock to certain countries.

Marek's disease is perhaps the most insidious virus the poultry industry faces. Significant success in the control of MD has been achieved through the use of vaccines that prevent tumor development—one of which became the first vaccine ever developed to prevent a cancer, but current vaccines do not block viral infection and spread. Scientists speculate that vaccine selection pressures have resulted in new highly virulent viral strains, which reportedly cause greater than 50 percent mortality in certain flocks. Continued reports of periodic MD outbreaks in vaccinated flocks worldwide—with increasing reports of vaccination breaks and emergence of more virulent pathotypes—point to the need for new strategies to control this re-emerging viral disease to prevent devastating losses in commercial layer and broiler flocks.

**Research Needs:**

The availability of genomic information from the chicken genome project and from sequencing the genomes of avian tumor viral strains provides new opportunities for understanding the genes and gene products associated with mechanisms of disease, for implementing genome-wide marker-assisted selection, breeding for disease resistance, and discovering highly effective vaccines for targeted animal populations. The implementation of a genomics-based research program will identify and decipher genetic and biological determinants of virulence, immune evasion mechanisms, and the emergence of new tumor viral strains. Emphasis will be given to understanding the host genetic determinants that influence mechanisms of avian tumor virus disease and protective immunity.

**Anticipated Products:**

- Scientific information on how genetic variations influence the immune response to Marek's disease infection.
- Scientific information on how the interplay between specific host and Marek's disease viral genes, and the variation within these genes, leads to disease susceptibility or resistance.
- Simple molecular tests to pathotype field strains of Marek's disease virus.
- Viral genes responsible for pathogenesis and identification of predictors of virulence shifts.
- Viral genes associated with immune evasion mechanisms.
- Scientific information on the biological pathways that lead to the development of Marek's disease.
- Vaccine-induced determinants of protective immunity.
- A safe and effective vaccine with mass vaccination capability that conveys protection against emerging Marek's disease viral strains in defined host animal genotypes.

**Potential Benefits:**

The availability of genomic-based countermeasures will provide new synergistic options that can be used strategically by the poultry industry to design effective control programs against emerging Marek's disease viral strains.

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

Highly infectious diseases include some of the most important foreign animal diseases but also many endemic pathogens affecting our intensive production of livestock and poultry. Highly infectious diseases often use mucosal surfaces as portals of entry that include the respiratory and/or gastrointestinal tracts of animals. These surfaces provide the gateway by which many pathogens of livestock and poultry gain entry into the host and are the site where the host first recognizes and reacts to foreign invaders. Pathogens in this category include several important viral, bacterial, and parasitic infections of the gastrointestinal and respiratory tracts. Many of these pathogens interact to form polymicrobial infections and disease complexes consisting of primary pathogens and secondary opportunistic invaders. Mucosal surfaces are the first line of defense against these pathogens, providing a physical barrier consisting of a single epithelial cell layer with overlying mucus, and sentinel cells that continuously sense the environment and coordinate defenses to protect mucosal tissues. Mucosal surfaces also represent the main sites of interaction between the host and environmental organisms—known as commensal organisms—that provide important benefits to the host such as nutrients and abundant immunostimulatory molecules. These host defense mechanisms play a central role in the control of adaptive immune responses, but the mechanisms of protective immunity remain for the most part unknown.

Highly infectious pathogens of livestock and poultry present exceptional opportunities for applying a genomics-based approach for understanding host-pathogen interactions and the genetic variations associated with infection, replication, tissue tropism, host-range specificity, transmission, and innate and adaptive immune responses. The goal of the research is to develop synergistic countermeasures for preventing and controlling important diseases of livestock and poultry. The research will focus on but will not be limited to the following highly infectious diseases: Porcine Reproductive and Respiratory Disease Syndrome (PRRS); Classical Swine Fever; Bovine Viral Diarrhea (BVD); Bovine Respiratory Syncytial Virus (BRSV); Avian Coccidiosis; Avian Influenza; and Foot-and-Mouth Disease.

**Research Needs:**

Many of the available tools for preventing and controlling these diseases are ineffective, and significant scientific gaps in our knowledge of immunology and genetic disease susceptibility have prevented advances in the discovery of effective countermeasures to prevent and control these pathogens in the field. Because many of these diseases are complex entities involving multiple pathogens and multiple disease resistance pathways, genomic approaches provide new opportunities for addressing issues of pleiotrophism and gene epistasis. Scientists

will interface high throughput genomic approaches with disease modeling studies in targeted animal populations to decipher genetic and biological determinants of disease susceptibility. These studies will lead to the discovery of innovative tools to prevent and control economic losses from important pathogens of livestock and poultry. It is critical that this research be aligned with research components focused on the pathogenic determinants of virulence, disease transmission, host range specificity, and tissue tropism (see Research Components 4, 5, and 6).

**Anticipated Products:**

- Genes that confer susceptibility to highly infectious diseases of cattle, pigs, and poultry.
- Genomic regions and specific structural variations that are associated with differences in pest- and disease-resistance traits.
- Methods of predicting genetic merit based on dense SNP-based marker data.
- Enabling tools (immunological reagents, SNP markers and SNP Haplotypes) to propel our understanding of host responses to mucosal diseases.
- Genes mediating resistance from previously determined quantitative trait loci (QTL) associated with susceptibility to diseases of livestock and poultry.
- Differences in host immunological and physiological responses to mucosal diseases associated with genetic markers and then fine-mapped to specific genes.
- Gene expression and genetic variations associated with protective mucosal immune responses identified and characterized.
- Biological determinants of innate and adaptive protective immunity identified and characterized.
- Comparative genetic maps to identify areas of similarity (synteny) between the bovine, swine, avian, humans, and mice genome.
- Genetic and biological markers for the detection of underlying disease infections.
- Highly effective diagnostics, vaccines, and biotherapeutics designed to prevent and control highly infectious diseases in targeted animal populations.

**Potential Benefits:**

- The ability to identify animals susceptible to mucosal pathogens likely to be encountered in defined production systems will enhance our ability to improve farm Biosecurity measures and the management of diseases.
- Animal genomics research will make important contributions to our understanding of developmental biology and advance human and veterinary biomedical research.
- Comparative animal genomics will provide new venues for understanding animal and human host responses to zoonotic diseases.
- Farm animal functional genomics will provide new research platforms for understanding wildlife diseases.

- Animal genomics will provide the tools to align animal health traits with targeted production environment to increase their performance in intensive or extensive management systems.
- Animal genomics will provide new technologies to enhance marker assisted selection of disease resistance traits.
- Animal genomics will provide new genomics-based diagnostic tools for managing disease susceptibility traits at the farm level.
- Targeted disease resistance to reduce mucosal infectious diseases will lessen the use of chemicals and drugs to control animal disease in the farm environment.
- Genetic markers will enhance traceability and on-farm Biosecurity.
- Enhance the ability to breed animals for selected traits that confer health and environmental sustainability in target management production system.
- Animal genomics will enable the design of highly effective diagnostics, vaccines, and biotherapeutics to prevent and control mucosal diseases in targeted animal populations.

### **Problem Statement 2C: Bovine Mastitis**

Mastitis continues to be the single most costly dairy disease, with economic losses approaching \$2 billion annually. Severe cases of clinical mastitis cause decreased milk yield, abortion, poor reproduction, and even death. A single case of clinical mastitis can cost up to \$200 due to mammary gland damage, loss of milk production, discarded milk, and the costs of treatment and labor. Significantly, more than half of the economic losses associated with mastitis are due to sub-clinical infections, which is often associated with an elevation in the number of somatic cells in the bulk tank of the dairy farm. (The National Mastitis Council calculated herd losses at 6 percent for bulk tank somatic cell count (BTSCC) at 500,000 cells/ml, and 18 percent for BTSCC at 1,000,000 cells/ml, when compared to herds with bulk tank cell counts 200,000 cells/ml or less.) Aggregated across all U.S. dairy cows, annual loss associated with sub-clinical mastitis would be approximately \$1 billion. Additional costs that are seldom mentioned are incurred by the processing industry in terms of reduced cheese yields and the manufacture of products with reduced shelf life and consumer acceptance.

Even with proper dry-cow therapy and proper sanitation, the best-managed herds will see 4 to 6 percent of quarters infected at calving. Mastitis is also among the top 3 reasons for culling cows. There are currently very few tools to effectively prevent or treat either environmental and coliform mastitis, or mastitis caused by select contagious pathogens such as *Staphylococcus*. The pathogen profile is herd dependent and often changes with time, making treatment even more difficult.

Antibiotics are often used to treat and prevent mastitis, but their use in food producing animals remains a major concern as continual exposure to antibiotics may pose human health risks. Also, currently approved antibiotics are largely ineffective against the most prevalent pathogens that cause clinical mastitis in cattle. Research has shown that intramammary antibiotic treatment may not be cost effective and may possibly be detrimental

to the cow's health. Importantly, organic milk producers have almost no options available to them for the prevention and treatment of mastitis.

This research will provide information to better understand the pathogenesis of mastitis and immuno-suppression in dairy cows. Emphasis will be placed on identifying the biomarkers and genetics of immune cells active in the onset and resolution of mastitis in cows, studying cases that are both infectious and non-infectious in nature causes as well as covering both the acute and chronic. In particular, the dynamics of immune cells and the host interactions responsible for bovine mastitis will be assessed, concentrating on understanding how these dynamics prevent bacterial control. Another important emphasis will be on the development of novel intervention strategies to enhance the repair of the mammary gland by directly impacting the embryonic stem cells. Using the strategies developed, researchers will also examine animals of determined genotype and phenotype and specific immune in attempt to gain genetic information that will lead to the prevention, control, and resolution of mastitis. This information, in turn, will be used to develop innovative immune-intervention strategies and immuno-modulators that may assist in controlling mastitis without antibiotics.

**Research Needs:**

The goal of the ARS mastitis research program is to develop genomic- and immunologic-based strategies to prevent and control bovine mastitis. By applying a functional genomics approach to understand variations in gene expression in bovine inflammatory embryonic stem cells in response to bacterial challenges of the mammary gland, researchers will provide a new understanding of gene interactions involved in immune cell activation, migration, and host responses.

**Anticipated Products:**

- Genetic variations associated with differences in immune cell activation, migration, and host responses to bacteria.
- New biotherapeutic platforms based on protective host proteins to induce and supplement the cow's innate immune response.
- Therapeutics to reduce cell damage and enhance repair during mastitis.
- Markers for mammary stem cells and methods for their regulation.
- New management and nutritional schemes to prevent metabolic stresses contributing to immunosuppressive states in the dairy cow.

**Potential Benefits:**

The development of new genomics- and immunologic-based strategies will provide dairy farmers with new and effective options for controlling mastitis.

**Problem Statement 2D: Increasing Gut Health and Disease Resistance**

There is a growing body of evidence showing that the microbial community in the gut helps balance the immune system and influences its host's development, fitness, and metabolism. Studies of the human gut microbiome suggest that the gut microbiota

influences the efficiency of calorie harvest from the diet and how these calories are used and stored. There is also accumulating evidence supporting the hypothesis that obesity and related metabolic diseases develop because of low-grade, systemic, and chronic inflammation induced by diet-disrupted gut microbiota. It has been shown that genotype, in combination with the gut microbiome, is linked to important physiological states, such as weight and disease. Thus, the outcome of a specific trait like feed efficiency may be influenced by interactions between an animal and its microbial community. Given the evidence that feed efficiency is affected by the gut microbial community, it is important to understand how the microbial community composition in animals with high and low feed efficiency might differ and to potentially determine whether a core set of microbes could be correlated with high or low feed efficiency animals. It could also be highly desirable to explore not only the differences in communities, but also how the genotype is correlated with gut microbial community composition and health. It may therefore be possible to construct a microbial community that could provide optimal feed efficiency and boost the level of disease resistance with a given animal genotype.

**Research Needs:**

Hundreds of beneficial microorganisms live in the gut of animals and play a key role in the development of the immune system, but very little is known about the identity of these microorganisms or the specific host-microorganism(s) interactions that promote health and confer disease resistance. The goal of this research program is to improve the gut health of farm animals to lower their susceptibility to diseases, increase disease resistance, and feed efficiency.

**Anticipated Products:**

- Scientific information on gut microbial composition and the relative abundance of commensal microbial species in livestock and poultry.
- Specific microbes that may account for differences between high and low feed efficiency individuals.
- Scientific information on the correlation between subclinical infections caused by certain enteric pathogens and feed efficiency.
- Diagnostic tests to identify animals with specific microbes associated with high or low feed efficiency.
- Feed additives and micronutrients that can improve feed efficiency, disease resistance, and health by promoting or inhibiting the growth of specific enteric microorganisms.
- Large-scale population studies to validate the effectiveness of preventive health management programs derived from the re-engineering of gut microbiomes using specially designed feed rations and diets.

**Potential Benefits:**

If one or more microbial species are found to be associated with differences in feed efficiency, this information could be utilized to account for previously unexplained variation and thereby increase the heritability of feed efficiency, which would more rapidly select for higher performing farm animals. The development of diagnostic tests could be used to identify animals with specific



microbes associated with high or low feed efficiency, including the response to specific enteric pathogens. This research will also enable the search for specific feed additives that can promote or inhibit the growth of specific microbes. Furthermore, it is highly possible that certain enteric pathogens may be a major factor in influencing the level of feed efficiency. Ultimately, the goal of this research is to identify specific animal genotype-microbial interactions that are correlated with high feed efficiency. The biological information from this research will identify how microbes, an “environmental factor,” influence feed efficiency and will help industry to more precisely select for or promote animals with high feed efficiency.

### **Problem Statement 2E: Genetics of Prion Disease Susceptibility**

Prion diseases have stimulated intense scientific scrutiny since it was first proposed that the infectious agent was devoid of nucleic acid. Despite this finding, host genetics has played a key role in understanding the pathobiology and clinical aspects of prion diseases through the effects of a series of polymorphisms and mutations in the prion protein gene. The advent of vCJD confirmed a powerful human genetic susceptibility factor, as all patients with clinical disease have an identical genotype at the polymorphic codon 129 of the prion gene. The alternative variant at codon 129 is not protective, however, and abnormal prions have been found in lymphoid tissues of individuals of other prion genotypes after exposure to transfused blood products from patients who later succumbed to the disease. Familial forms of prion diseases are also known to be inherited in an autosomal dominant pattern, which means one copy of the altered gene in each cell is sufficient to cause the disorder. In most cases, an affected person inherits the altered gene from one affected parent. In some people, familial forms of prion disease are caused by a new mutation in the prion gene. Although such people most likely do not have an affected parent, they can pass the genetic change to their children. Familial Creutzfeldt-Jakob disease (fCJD), Gerstmann-Sträussler-Scheinker (GSS) syndrome, and fatal familial insomnia (FFI) represent the core phenotypes of genetic prion disease.

Genetic studies in animals have uncovered similar polymorphisms and mutations in the prion protein gene. Genetic information has led to the discovery of genotypes with relative susceptibility and resistance to Scrapie in sheep. Current Scrapie control programs in the United States and Europe are based on the elimination of susceptible genotypes from the breeding pool. The 2006 U.S. H.-type atypical BSE cow had a polymorphism at codon 211 of the bovine prion gene, resulting in a glutamic acid to lysine substitution (E211K). This substitution is analogous to a human polymorphism associated with the most prevalent form of heritable TSE in humans, and it is considered to have caused BSE in the 2006 U.S. atypical BSE animal.

### **Research Needs:**

The functional genomics of disease resistance are not completely understood, and recent research suggests genetic variations may lead to different clinical outcomes (e.g., vCJD, atypical BSE, atypical Scrapie). This research area is aimed at utilizing powerful computational biology and bioinformatic approaches, along

with traditional animal breeding experiments, to steadily improve our understanding of mechanisms of genetic disease resistance.

**Anticipated Products:**

- Genetic variations associated with disease susceptibility.
- Scientific information on the correlation between host genotypes and the phenotypes of prion agents.
- Genetic factors controlling susceptibility of goats to sheep Scrapie.
- Scientific information to evaluate the effectiveness of disease resistance breeding programs in sheep.
- Scientific information to evaluate sheep ARR/ARR genotype for resistance to different TSE strains.
- Scientific information on the influence of genetics on BSE incubation time and the frequency of animals carrying the E211K allele.

**Potential Benefits:**

These studies will yield critical genetic information that influences disease susceptibility, clinical outcomes, surveillance programs, and the discovery of diagnostic techniques as well as preventative and treatment programs.

**Component 2 Resources:**

The following ARS locations have research projects addressing the problem statements identified under Component 2:

- Ames, IA
- Clay Center, NE
- Pullman, WA
- East Lansing, MI
- Beltsville, MD
- Athens, GA

**Component 3: Zoonotic Diseases**

Zoonotic diseases, by definition encompassing all infectious diseases transmitted from animals to man, represent one of the leading causes of illness and death in people. In developing countries, zoonotic diseases stand out as the most prevalent and important threat to public health. Zoonoses also have a negative impact on commerce, travel, and economies worldwide. In industrialized nations, zoonotic diseases are of particular concern to the agricultural sector. 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 must play an important role in preventing these diseases from propagating in domestic animals, starting with proper surveillance systems. Over the years, USDA has invested significant resources in attempts to eradicate endemic zoonoses from livestock populations in the United States (e.g., brucellosis and

tuberculosis). However, their persistence in wildlife reservoirs continues to pose challenges. 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 their animal source and protect our Nation from these important public health threats.

The ARS zoonotic bacterial diseases research program focuses on brucellosis, leptospirosis, and tuberculosis with the strategic goal of developing countermeasures to prevent disease transmission in domestic livestock and wildlife reservoir hosts. Zoonotic viral 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 addressed under Component 6B (Hemoparasitic Diseases) and Component 7 (BSE).

According to rankings at the March 2010 Animal Health Program Planning Workshop, zoonoses are the 2<sup>nd</sup> priority of the swine industry, 1<sup>st</sup> of the wildlife industries, 3<sup>rd</sup> of the dairy industry, and 2<sup>nd</sup> of the beef industry.

### **Problem Statement 3A: Brucellosis**

Brucellosis is one of the most significant zoonotic diseases of livestock worldwide. It is a devastating infectious disease that causes significant morbidity and mortality in animals and man. *Brucella* species are also an important cause of abortion in cattle, small ruminants such as sheep and goats, and swine, resulting in dire economic losses once a herd becomes infected. Brucellosis has been subject to an intensive eradication campaign in the United States for the last 50 years. Certain *Brucella* species tend to have a predilection for certain animal species (e.g., *B. abortus*-cattle, *B. suis*-pigs, *B. melitensis*-sheep and goats) but many can infect all mammals with varying degrees of virulence. Wildlife species can be an important source of infection; for example, bison and elk in Yellowstone Park, and more recently feral swine. Most importantly, brucellosis has been classified by the U.S. biodefense community as a potential agent for bioterrorism due to its ability to be weaponized.

Tremendous strides have been made in eradicating brucellosis from U.S. cattle and domestic swine herds. The annual losses from brucellosis including lowered milk production, aborted calves and pigs, and reduced breeding efficiency have gone from more than \$400 million in 1952 to less than \$1 million today. Studies have shown that if the U.S. brucellosis eradication program efforts were stopped now, the costs of producing beef and milk would increase by an estimated \$80 million annually in less than 10 years. Nevertheless, brucellosis has been a tremendous burden for U.S. livestock producers, and success has been costly. For example, in 2004, the Federal government withdrew the State of Wyoming's brucellosis-free status after finding animals in two herds infected with brucellosis, and new emergency Federal requirements for mandatory testing were put into place to prevent interstate transmission of brucellosis. The costs of additional testing and the loss in livestock sales were estimated to be as high as \$25 million over a seven year period. Over the years, Federal and State governments, along with the livestock industry, have spent billions of dollars to control and eliminate brucellosis.

Recently, feral pigs have become an important source of brucella infection for dairy herds in Texas and Florida, as feral pigs gain access to dairy farms and infect the cattle with *B. suis*. The incidence of *B. suis* in feral pigs has been as high as 37 percent in some areas. Culturing the organism is currently the only way to differentiate the type of brucella with which an animal is infected. Because brucella is the most common organism associated with inadvertent laboratory infection it is important to improve our diagnostic capabilities.

A serious problem in much of the world where sheep and goats are important in agriculture, *B. melitensis* is poorly understood in terms of its incidence and epidemiology in U.S. sheep and goats. As the organic farm markets increase, milk from sheep and goats is increasing in popularity and is often not pasteurized, making it a risk to human health. Thus, it is of increasing importance to both human and animal health that researchers determine the incidence and epidemiology of the organism, and develop control strategies and differential diagnostic assays.

As we continue a long and arduous brucellosis eradication campaign, better diagnostic measures are needed to find the last reservoirs of infection in our domestic livestock. In addition, the significance of wildlife reservoirs as the source of new infections cannot be underestimated, and the need is ever present for effective countermeasures designed specifically to control brucellosis in wildlife.

**Research Needs:**

Genomic analyses of *Brucella* species are needed to identify unique sequences to further the development of serological diagnostic tests that can differentiate the various *Brucella* species without culturing. Research is also needed to create new vaccines and diagnostic assays to control infection and transmission specifically designed for the control and eradication of brucellosis in wildlife. The new vaccine strategies will include developing oral vaccines that would be economical and reliable for use in wildlife reservoirs.

**Anticipated Products:**

- Scientific information to differentiate brucella species by determining the unique sequences associated with phenotypic variations in virulence, host range, and persistent infections that will support diagnostic and vaccine discovery research initiatives.
- Pathogenesis studies of *Brucella* species that will include identifying mechanisms of persistent infections, host tolerance, and protective immunity.
- Diagnostic and intervention strategies for bison, elk, and feral swine that will enable control and potentially eradication in the wildlife reservoir.
- Diagnostic and intervention strategies for *B. melitensis* that will ensure a safe food supply and goat and sheep health.
- New vaccine platforms, with an emphasis on oral delivery, designed to control and eradicate brucellosis in bison, elk, and feral swine.

**Potential Benefits:**

The discovery of new countermeasures specifically designed to prevent and control brucellosis in wildlife will eliminate new source of infections and enable the eradication of brucellosis in our domestic livestock.

**Problem Statement 3B: Leptospirosis**

Leptospirosis is a zoonotic disease of increasing global importance. It is primarily an occupational disease of farm workers, veterinarians, and slaughter plant workers, but can infect most animal species. Rodents, rats, and mice composed half of the reported source of infection, but almost 40 percent were from farm animals and companion animals. Case fatality rates can be 20 percent or higher. Importantly, *Leptospira* can be acquired directly from the environment, which places it in the category of diseases that cannot be eradicated. It can have significant and widespread economic impact due to the international trade of animals and semen, high treatment and control costs, and reduced milk yields and reproductive failures.

In spite of its significant medical and economic impact, Leptospirosis is one of the most overlooked and neglected diseases because it is very difficult to diagnose, and most endemic countries (including the United States) lack a notification system. The microscopic agglutination test (MAT) is the “gold” standard in diagnostic testing of Leptospirosis; however, this test is slow, difficult to standardize, requires live *Leptospira* cultures, and is vulnerable to interpreter variations. The development of new diagnostic technologies is therefore a top priority.

Recent global epidemiological surveys indicate that the most prevalent serovar is Icterohaemorrhagiae, followed by Pomona, Sejroe, Australis, Autumnalis, Grippotyphosa, and Canicola. The most prevalent and important serovars in U.S. livestock are Hardjo and Pomona, which cause maintenance host infections in cattle and pigs, respectively, and play an important role in the transmission. Hardjo and Pomona are often associated with late term abortions, but can also cause subclinical disease that can have significant effects on herd reproductive performance. The primary control method is whole herd vaccination, whose efficacy is dependent on having the correct serovar in the vaccine, as very little cross-protective immunity is provided with existing commercial vaccines.

**Research Needs:**

Research will be conducted to characterize spirochete strains associated with field outbreaks, determine how these bacteria interact and elicit host responses during infection, and determine 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) will be extended to other *Leptospira species* for comparative microbial genomics studies that will lead to the identification of unique sequences to support diagnostic and vaccine discovery research programs. Microarray technology will be used to measure *Leptospira* gene expression changes in incidental versus maintenance host infections. A genomics-based approach will be used to assess

mutant strains with defined phenotypic characteristics and analyze how these mutants interact with the host and alter global patterns of gene expression. Diagnostic tools will be developed to support molecular epidemiology studies to understand the ecology of *Leptospira species* and serovars. Host-pathogen interactions will be assessed to determine genomic variations associated with persistence infections of reservoir hosts.

#### **Anticipated Products:**

- In vitro disease models consisting of host cell cultures that will lead to the molecular characterization of host-bacterial interactions, variations in gene expression, and associated pathogenic mechanisms.
- Scientific information on the protective immune responses to spirochete antigens in large and small animal disease models.
- Large-scale sequence analyses to characterize the genome of selected spirochetes and identify strain-specific regions in various *Leptospira* strains.
- Scientific information on the genetic variability of key genes using multi-locus sequencing techniques.
- Transcription studies to identify differentially expressed genes to characterize virulence traits and select vaccine candidates.
- Genetically altered bacteria for *in vitro* and *in vivo* studies to establish key links between specific genes and phenotype.
- Efficacious molecular vaccines to prevent the spread of Leptospirosis in domestic animals and wildlife.

#### **Potential Benefits:**

- The functional genomics analysis of *Leptospira* strains will enable us to identify virulence determinants that will lead to vaccine discovery research and new diagnostic platforms for classification of field strains.
- Developing new generation vaccines will improve the control of maintenance and accidental host infections in our domestic animals thereby lowering the incidence of disease and protecting farm workers from spirochete-associated zoonoses.

#### **Problem Statement 3C: Tuberculosis**

Before eradication efforts were initiated in the United States in 1917, bovine TB (caused by *Mycobacterium bovis*) was a disease of epidemic proportions. Implementation of strict test and cull procedures and mandatory pasteurization of milk has significantly reduced *Mycobacterium bovis* as a cause of TB in the human population. Until recently, the eradication program has been based on abattoir inspections, testing, and depopulation of infected herds, efforts that have been largely successful, reducing the reactor rate in cattle from about 5 percent to currently less than 0.02 percent. From 1917 to 1962, the USDA tuberculosis eradication campaign cost \$3 billion in 2003 dollars but resulted in an estimated annual savings of 12 times this cost in decreased carcass condemnation and improved animal productivity. Moreover, by reducing the number of cattle lost to tuberculosis, it is estimated that the program saves \$150 million per year in replacement costs alone.

However, even as the incidence of tuberculosis in the United States declines, Federal and State control programs are facing new challenges. In 1994, a white-tailed deer from northeastern Michigan was found to be infected with *Mycobacterium bovis*. Since then, testing has found the presence of TB in both the white-tailed deer population as well as cattle in the affected area. To date, over 650 cases of *Mycobacterium bovis* infection has been identified in deer, and 49 positive cattle herds have been identified. Control strategies have failed to eradicate TB from either population in Michigan. *M. bovis* infection has recently been detected in 27 deer and 12 cattle herds in Minnesota, and has been confirmed in Nebraska, Indiana, North and South Dakota, New Mexico, and California, with detection rates increasing on a regular basis. Another source of *M. bovis* in the United States is imported infected cattle from other countries, particularly Mexico. In addition, infections in captive deer and elk herds and the presence of tuberculosis in zoo and wildlife species maintained for exhibition remain problematic in eradicating *M. bovis*. The detection of tuberculosis in 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, the Animal and Plant Health Inspection Service (APHIS) has requested that ARS redirect its tuberculosis research efforts to examine alternatives to abattoir inspections and test and slaughter campaigns. Specific needs 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 *Mycobacterium bovis* in infected herds.

Stakeholders at the April 2010 Animal Health Research Planning Workshop identified tuberculosis as the 2<sup>nd</sup> priority of the beef industry, 3<sup>rd</sup> priority of the dairy industry, and top priority of specialty farm animal species. Further, stakeholders from the beef industry identified the following research priorities: development of diagnostics and vaccines and management strategies to control the spread of *M. bovis* at the wildlife / livestock interface.

**Research Needs:** The ARS bovine tuberculosis research program will include the identification of microbial immunogens critical for the induction of protective immunity. The genome of common environmental mycobacteria will be sequenced to develop assays that differentiate these organisms, which often result in false positive responses. Variations in *Mycobacterium bovis* infections, pathogenesis, and immune responses will be characterized in domestic livestock and relevant wildlife reservoir hosts. Improved diagnostic and vaccine countermeasures specifically designed for the control and eradication of *Mycobacterium bovis* will be discovered.

**Anticipated Products:**

- Microbial immunogens critical for development of protective immunity.
- Scientific information to increase our understanding of the molecular pathogenesis of *Mycobacterium bovis* infections.

- Comparative analyses to understand the variations of host immune responses to natural infections versus vaccination as well as neonatal versus adult cattle responses.
- Improved sensitive and specific diagnostic platforms amenable to the rapid screening of large cattle herds.
- Diagnostic platforms to differentiate infected versus vaccinated animals.
- Effective vaccine platforms to prevent and control *Mycobacterium bovis* in cattle and relevant wildlife reservoir hosts.

**Potential Benefits:**

New, improved countermeasures to control *Mycobacterium bovis* in wildlife and domestic livestock will help prevent new incidences of bovine tuberculosis and support its eradication from the United States.

**Component 3 Resources:**

The following ARS locations have research projects addressing the problem statements identified under Component 3:

- Ames, IA

**Component 4: Respiratory Diseases**

Endemic respiratory diseases are the primary health threat of livestock and poultry raised in intensive production systems. The costs of respiratory diseases are significant and disease outbreaks often determine the difference between profit and loss. 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 the majority of livestock and poultry being vaccinated for a number of primary pathogens associated with respiratory disease today, lesions are still prevalent at slaughter and their impact on weight gain and carcass quality is significant. Respiratory diseases continue to be a major problem today, in spite of decades of control measures using antibiotics and vaccines. Important scientific gaps remain in our understanding of respiratory pathogen complexes and the ecological and host interactions that lead to disease and production losses. 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 at our April 2010 Animal Health Workshop representing the beef industry ranked respiratory diseases as their 1<sup>st</sup> priority, while the poultry broiler industry ranked it



their 4<sup>th</sup> priority, and the swine industry their 4<sup>th</sup> priority. Stakeholders from the swine industry ranked complex disease interactions, including respiratory diseases, as their 4<sup>th</sup> priority, but they ranked porcine reproductive and respiratory syndrome virus and re-emerging diseases, such as swine influenza, as their 1<sup>st</sup> and 3<sup>rd</sup> priorities, respectively, both of which are major contributors to the porcine respiratory disease complex. Because of the sheer number of pathogens involved in respiratory diseases and the ability of many pathogens to cross the species barrier, ARS will use available resources to focus strategically on priority respiratory pathogens associated with the bovine, porcine, and poultry respiratory disease complexes. Emphasis will be 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.

#### **Problem Statement 4A: Bovine Respiratory Diseases**

Respiratory disease of beef cattle is the most costly disease facing producers today, estimated to cost \$3 billion to the U.S. cattle economy annually, and is among the most costly diseases for dairy, sheep and goat producers. The United States has the largest fed-cattle industry in the world, and is the world's largest producer of beef, primarily high-quality, grain-fed beef for domestic and export use. Cash receipts from marketing cattle and calves were \$47.3 billion in 2004, by far the largest U.S. agricultural commodity in terms of cash receipts, with sales of 53.8 billion pounds. Recent NAHMS surveys confirm that respiratory disease continues as the leading cause of morbidity and mortality in U.S. feedlots and is the most common cause of weaned dairy heifer mortality. This represents the single largest variable cost of cattle production that can be controlled by the producer. As such, the availability of effective countermeasures to prevent and control disease threats will have a significant impact on the future of the industry.

The nature of cattle production and marketing in the United States, however, produces an exceptional challenge to efforts directed at disease control. Movement of cattle from cow-calf operations to stockers to feed yards increases stress and provides high levels of exposure to numerous infectious agents. Countermeasures, such as vaccines or biotherapeutics, must therefore be rapidly and highly effective. Antibiotic usage for prevention and therapy of respiratory disease is widespread and very costly to producers, but is not sustainable. Regulatory agencies will continue to limit the availability of these controversial tools used to raise cattle and will continue to raise safety standards. A variety of vaccines to a number of respiratory pathogens are commercially available and widely used to mitigate the effects of several significant viral pathogens, though additional viruses not present in current vaccines are playing a role in disease pathogenesis. Vaccines against the major bacteria are also numerous but are used less because of their more limited efficacy in field situations. Increasing our understanding of disease threats and the discovery of countermeasures specifically designed to control and prevent disease introductions will be critical to sustain the efficiency of the U.S. cattle industry.

Stakeholders representing the beef industry at the April 2010 Animal Health Workshop identified bovine respiratory diseases as their 1<sup>st</sup> priority.

**Research Needs:**

Stakeholders from the beef industry identified the following research priorities: the host-pathogen interaction and their ability to act in synergy with other factors, altered host responses by primary pathogens and management practices, novel preventatives and therapeutics, and surveillance for pathogens and host factors for early intervention.

The ARS bovine respiratory diseases research program will include the development of improved diagnostic tests to enable the rapid detection of respiratory pathogens on premises. The pathogenesis and polymicrobial interactions of respiratory pathogens will be investigated. New innovative prevention and control strategies for ruminant respiratory diseases will be discovered. Research will be conducted to define mechanisms of disease transmission of respiratory pathogens in relevant beef production systems. Epidemiological studies will also be conducted to identify reservoirs of respiratory pathogens. Host responses to respiratory pathogens, including mechanisms of immune evasion and protective immunity will be characterized.

**Anticipated Products:**

- Discovery of determinants of virulence and characterization of mechanisms of infection.
- Scientific information on pathogen interactions that lead to polymicrobial infections and respiratory disease complexes.
- Scientific information on mechanisms of immune evasion and protective immunity.
- Drug and vaccine delivery systems that target the ruminant respiratory tract.
- Alternatives to antibiotics for preventing and treating respiratory diseases.
- Diagnostic platforms that can be used to develop rapid on-site tests.
- Highly effective vaccines that induce targeted immune responses to prevent colonization of the respiratory tract and prevent shedding and disease transmission.

**Potential Benefits:**

The overall impact of the research will be improved diagnosis, control, and prevention of endemic respiratory diseases that will benefit the beef industry. The impact of the research will be derived from the identification of disease pathogen reservoirs, understanding pathogen transmission, and the discovery and technology transfer of highly effective diagnostics, vaccines, and biotherapeutics designed to control and eradicate respiratory diseases from herds. Incremental development of these tools will provide more predictable costs and better potential returns to cattle and sheep producers, making the business of livestock production sustainable. The overall goal of these projects is to produce scientific

information and tools that will enable the U.S. beef industry to remain competitive and profitable.

#### **Problem Statement 4B: Porcine Respiratory Diseases**

The U.S. swine industry is the 3<sup>rd</sup> largest producer of pork in the world, marketing 27.8 billion pounds of pork and having cash receipts totaling \$14.3 billion in 2004. The United States is also the 2<sup>nd</sup> largest consumer and exporter of pork and pork products. Pork accounts for about 1/4 of domestic meat consumption and about 1/2 of the meat consumed world-wide. The U.S. herd is approximated at 60 million, and the industry is becoming more and more industrialized and integrated. According to the 2000 NAHMS survey, respiratory disease was the single greatest cause of mortality in swine, accounting for 28.9 percent of nursery deaths and 39.1 percent of deaths in grower/finisher pigs. The National Pork Board has consistently listed the porcine respiratory disease complex (PRDC) as a top research priority.

Respiratory disease in swine, as with other species, is generally considered multi-factorial, caused by a combination of viral and bacterial infectious agents, as well as adverse environmental conditions. The list of infectious agents that cause respiratory disease in swine is extensive and includes both viral agents, such as porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), Porcine circovirus type 2 (PCV2), and porcine respiratory coronavirus (PRCV); and bacterial agents, such as *Mycoplasma hyopneumoniae*, *Bordetella bronchiseptica*, and *Pasteurella multocida*. Although any one of these pathogens can potentially cause disease on its own, more serious and chronic respiratory disease results, and more economic losses are incurred, when infection with multiple pathogens occurs. Although the multi-factorial nature of respiratory disease is well accepted, the specific mechanisms by which respiratory pathogens interact with each other or the host to cause more severe disease are poorly understood. A complete characterization and understanding of the molecular mechanisms underlying the pathogenesis of PRDC is critical in order to discover and develop effective countermeasures to control and eradicate this disease.

Stakeholders representing the pork industry at the March 2010 Animal Health Program Planning Workshop ranked PRRSV (see Component 2) as their 1<sup>st</sup> priority; emerging and zoonotic diseases, including SIV and MRSA, their 2<sup>nd</sup> priority; optimizing the health of growing pigs, which includes polymicrobial infections, microbial genomics, vaccines and diagnostics, as their 3<sup>rd</sup> priority; and healthy pig production with restricted antimicrobial access as their 5<sup>th</sup> priority.

#### **Research Needs:**

Four of the six priorities listed at the March 2010 workshop included polymicrobial infections or pathogens that directly impact the respiratory tract. Some of the pathogens listed are not directly linked to the respiratory tract; however, research on emerging and zoonotic bacteria will be conducted as necessary. The pathogenesis and polymicrobial interactions of respiratory pathogens will be investigated. Research will be conducted to define mechanisms

used by swine respiratory pathogens to cause disease by identifying and characterizing changes in gene expression of both the porcine host and the bacterial respiratory pathogens during the infectious process. Host responses to respiratory pathogens, including mechanisms of immune evasion and protective immunity will be characterized. New innovative prevention and control strategies for swine respiratory diseases will be discovered.

**Anticipated Products:**

- Scientific information on the pathogen interactions that lead to polymicrobial infections and respiratory disease complexes in swine.
- Scientific information on changes in gene expression underlying porcine immune responses to infection with respiratory pathogens.
- Scientific information on global changes in gene expression of porcine bacterial pathogens in response to respiratory infection.
- Discovery of determinants of virulence and characterization of mechanisms of infection.
- Scientific information on microbial genetic variations associated with differences in virulence and disease transmission.
- Scientific information on mechanisms of immune evasion and protective immunity.
- Efficacious vaccines that induce targeted immune responses to prevent colonization of the respiratory tract and prevent shedding and disease transmission.

**Potential Benefits:**

The overall impact of the research will be improved control and prevention of endemic respiratory diseases that will benefit the swine industry. The expertise on bacterial infections in swine will also be used to address new and/or emerging zoonotic bacterial diseases, such as MRSA. The impact of the research will be derived from better understanding the pathogenesis of disease and the discovery and technology transfer of control measures, including vaccines, designed to eradicate respiratory diseases from swine herds. The overall goal of these projects is to produce scientific information and tools that will enable the U.S. swine industry to remain competitive and profitable.

**Problem Statement 4C: Poultry Respiratory Diseases**

The U.S. poultry industry is the most productive worldwide with 34 million metric tons of broiler meat produced in 2007. The industry is fully integrated and supports the intensive production of 8.9 billion birds annually. However, endemic respiratory diseases continue to decrease the profitability of commercial poultry production. As we enter the 21<sup>st</sup> century, the single most important issue for poultry farmers will be how to lessen the direct and indirect cost of disease. A threshold has been reached where poultry farmers will have to either produce more birds to overcome the current cost of disease or produce the same amount of birds at a lesser cost with value-added disease control measures. As such, the availability of effective countermeasures to prevent and control disease threats will have a significant impact on the future of the industry. Regulatory agencies will

continue to limit the availability of controversial tools used to raise poultry and will continue to raise safety standards. The loss of antibiotics and the lack of alternatives is already affecting the cost of poultry production and increasing the urgency of controlling infectious diseases. More than 64 billion doses of poultry vaccines are produced annually, and their extensive use has benefited the poultry industry's profit margin. Nevertheless, most of the vaccines produced today are bacterin-based or live attenuated vaccines for the sole purpose of lessening clinical disease. Innovative vaccines and biotherapeutics with significant technological advancements have yet to be developed. Increasing our understanding of disease threats and the discovery of countermeasures specifically designed to control and prevent disease introductions will be critical to sustain the efficiency of the U.S. poultry industry.

The incidence, prevalence, and etiology of poultry respiratory disease pathogens are typically dependent on the specific industry segment and, with respect to chickens, the breed. The broiler, layers, and turkey producers report different respiratory disease pathogens as being most problematic. A 2009 Poultry Report compiled from a number of poultry organizations, including the American Association of Avian Pathologists (AAAP), Western Poultry Disease Conference, and North Central Avian Disease Conference, listed high-impact respiratory disease pathogens for the broiler industry as infectious bronchitis virus and laryngotracheitis virus; the layer industry expressed concerns with mycoplasmas, avian influenza, and infectious bronchitis virus; whereas the turkey industry identified loss of antibiotics and the potential impact of their loss on diseases of critical concern. Diseases of concern to the turkey industry include *Escherichia coli* (*colibacillosis*), *Ornithobacterium rhinotracheale* (ORT), *Bordetella avium* (turkey coryza), and influenza viruses, both avian and swine.

Stakeholders at our September 2010 Animal Health Workshop representing the poultry industry characterized the following respiratory pathogens as priority diseases: infectious bronchitis, infectious laryngotracheitis, turkey rhinotracheitis (TRT), 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*).

**Research Needs:**

Research will be conducted to define mechanisms of disease transmission of respiratory pathogens in relevant poultry production systems. Epidemiological studies will be conducted to identify reservoirs of priority respiratory pathogens. Host responses to respiratory pathogens, including mechanisms of immune evasion and protective immunity will be characterized. Researchers will develop improved diagnostic capabilities that will enable rapid differential diagnosis of respiratory pathogens on poultry farms. The pathogenesis and polymicrobial interactions of respiratory pathogens will be investigated. New innovative prevention and control strategies for poultry respiratory diseases will be discovered.

**Anticipated Products:**

- Scientific information on the characteristics of aerosol spread for priority respiratory pathogens in relevant poultry production systems.
- Discovery of determinants of virulence and characterization of mechanisms of infection.
- Scientific information on pathogen interactions that lead to polymicrobial infections and respiratory disease complexes.
- Scientific information on mechanisms of immune evasion and protective immunity.
- Drug and vaccine delivery systems that target the avian respiratory tract.
- Alternatives to antibiotics for preventing and treating respiratory diseases.
- Differential diagnostics platforms that can be used to develop flock-side tests.
- Highly effective vaccines that induce targeted immune responses to prevent colonization of the respiratory tract and prevent shedding and disease transmission.

**Potential Benefits:**

The overall impact of the research will be improved diagnosis, control, and prevention of endemic respiratory diseases that will benefit the poultry industry. The impact of the research will be derived from the identification of disease pathogen reservoirs, understanding pathogen transmission, and the discovery and technology transfer of highly effective diagnostics, vaccines, and biotherapeutics designed to control and eradicate respiratory diseases from poultry flocks. The overall goal of these projects is to produce scientific information and tools that will enable the U.S. poultry industry to remain competitive and profitable.

**Component 4 Resources:**

The following ARS locations have research projects addressing the problem statements identified under Component 4:

- Athens, GA
- Fayetteville, AR
- Mississippi State University, MS
- Ames IA
- Pullman, WA

**Component 5: Enteric Diseases**

Enteric diseases affect animals and humans universally and are the cause of significant production losses and mortality. Several 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, 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 disease research program will use available resources to focus on two major initiatives: 1) Johne's disease and 2) enteric diseases of poultry.

Stakeholders at our March 2010 Animal Health Workshop representing the dairy industry ranked Johne's disease as their 2<sup>nd</sup> priority; and stakeholders representing the poultry broiler industry ranked enteric diseases as their 2<sup>nd</sup> priority, with Coccidian and Clostridial diseases being the most important.

#### **Problem Statement 5A: Johne's Disease**

Johne's disease (paratuberculosis) is a chronic, progressive enteric disease of domestic and wild ruminants caused by infection with the intracellular pathogen, *Mycobacterium avium* subsp. *paratuberculosis*. It is estimated that 20-30 percent of U.S. dairy and cattle herds are infected with this organism. Johne's disease adversely affects the intrastate and interstate shipment of cattle as well as international exports, causing an excess of \$1 billion annually in lost revenue to our livestock industry. Cattle become infected as calves but do not develop clinical signs such as diarrhea, weight loss, and protein-losing edema until 2 to 5 years of age. During the protracted subclinical infection, infected animals are asymptomatic and shed the microorganism intermittently. Further complicating diagnosis, host immunity to infection is mediated by Th1-type responses in early infection, with a shift to Th2-type responses in later infection, precluding the use of a single diagnostic tool to accurately detect infection. Further, current vaccines do not prevent infection but only allay the more severe clinical signs of disease. Given the marginal tools for diagnosis and control of this disease, the incidence of Johne's disease (paratuberculosis) will continue to increase in United States.

#### **Research Needs:**

Completion of the sequencing of the *M. 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* will be conducted 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 will be conducted to ascertain potential mechanisms that contribute to the switch in Th1- to Th2-mediated immunity. Unique microbial genomic sequences and host responses will be used to implement a technology-driven vaccine discovery program.

**Anticipated Products:**

- 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.
- Highly effective vaccine platform that prevents subclinical disease, shedding of *M. paratuberculosis*, and progression to clinical disease.

**Potential Benefits:**

These studies will provide information on key host-pathogen responses during the infection process, leading to the development and application of genomic-based diagnostic tests and vaccines to prevent and control Johne's disease.

**Problem Statement 5B: Enteric Diseases of Poultry**

Enteric diseases remain a threat to the poultry industry, and countermeasures to prevent and control them are needed. Priority poultry enteric diseases include poult enteritis mortality syndrome (PEMS), poult enteritis complex (PEC), runting-stunting syndrome of broilers (RSS), Necrotic enteritis (*Clostridium perfringens*), as well as unclassified enteric diseases. PEMS affects young turkeys and is probably the most severe form of enteric disease.

The etiology or pathogens responsible for many enteric diseases are unknown, and determining the cause of enteric disease in poultry has been difficult. First, definitive identification of pathogens has been challenging as many enteric viruses cannot be grown in the laboratory, and available virus detection tests have poor sensitivity and specificity. Second, enteric diseases can be caused by two or more infectious agents, and numerous agents and combinations of agents cause clinically similar conditions. With the recent development of molecular detection methods, several different viruses have been identified as causes of gastrointestinal tract infections in poultry, including rotaviruses, coronaviruses, enteroviruses, adenoviruses, astroviruses, and reoviruses. In addition, a number of other viruses of unknown importance have been associated with gastrointestinal diseases in poultry, based on electron microscopic examination of feces and intestinal contents. Viral infections of the gastrointestinal tract of poultry are known to negatively impact poultry production.

**Research Needs:**

There is a need to understand and characterize the immune response during enteric infections and apply immunological and genomic approaches to identify host and pathogen genes involved in resistance to enteric infections. Tools are needed to study the epidemiology and genetic relationships of enteric infectious organisms and the processes that regulate host response to enteric infection to enable the development of effective strategies to prevent enteric diseases. Understanding the differential expression of genes that govern the processes



involved in host defense against enteric diseases will lead to novel interventions for the prevention and treatment of enteric infections such as diagnostics and vaccines and cost-effective Biosecurity control measures.

**Anticipated Products:**

- Discovery of quantifiable factors associated with disease risk.
- Discovery of modulators of stress in production systems that affect enteric disease development.
- Cytokines and expression profiles associated with processes involved in host defense during enteric infection.
- Discovery of 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.
- Intervention strategies that enhance the clearance of enteric pathogens.
- Immunointervention strategies that prevent the development of enteric infections.

**Potential Benefits:**

The ability to detect and determine the pathogens responsible for enteric disease complexes is the first step in any disease control program. In addition, understanding of the relationship of enteric pathogens to each other and their hosts will provide critical scientific information to support disease management programs. Importantly, this research will lead to the discovery and development of tools to enable the prevention and control of enteric diseases of poultry.

**Component 5 Resources:**

The following ARS locations have research projects addressing the problem statements identified under Component 5:

- Athens, GA
- Beltsville, MD
- Ames, IA

**Component 6: Parasitic Diseases**

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 these parasitic diseases, which cause significant losses in animal production due to lower weight gain, anemia, diarrhea, and death from parasites. For example, the control of nematode infections in cattle costs beef producers over \$1 billion per year. Moreover, many parasites are invasive and exotic to the United States and impact international trade. Most importantly, the emergence of drug resistant parasites against many commonly used pharmaceutical drugs has huge economic implications. To further complicate control, the populations of parasites may change with the climate changes anticipated with global warming.

Stakeholders at the March 2010 Animal Health Planning Workshop representing the equine industry ranked diseases that impact international movement of horses such as Piroplasmosis (Equine Babesiosis) as their 1<sup>st</sup> priority, and the goat industries also ranked internal parasites as its 1<sup>st</sup> priority. The poultry broiler industry identified enteric diseases such as coccidiosis its 3<sup>rd</sup> priority.

### **Problem Statement 6A: Gastrointestinal (GI) Parasitic Disease**

Gastrointestinal parasites of most species of domestic animals were until recently considered a minor health problem to their host. With the development of efficacious anti-parasitic drugs and strategies, most producers were confident that their parasite problems were controlled. In addition, the species of parasites impacting domestic agricultural animals had been stable for a long time. With the concern about global climate change, however, producers are worried that new parasites will enter the U.S. animal populations.

Currently, drug resistance has emerged as the single most important problem confronting the control of parasites in livestock worldwide. The use of drugs continues to be the primary treatment against parasites, but the intensive use of these products has resulted in some degree of resistance to the majority of the drugs currently available. A survey co-jointly conducted by the Food and Agriculture Organization (FAO) of the United Nation and the World Animal Health Organization (OIE) determined that more than 20 percent of the countries surveyed reported problems with drug resistant parasites.

The availability of effective drugs to control parasitic diseases in cattle and sheep in the United States is no less important. Helminthic diseases of cattle and sheep are rising in prevalence due to the ever-increasing incidence of drug resistance in parasitic nematodes. In swine, returning to agricultural practices where animals are frequently outdoors—with greater exposure to a variety of pathogens found in soil—increases the incidence of parasites and the potential for drug resistance. Developing control measures against nematodes will require knowledge of the species composition and the ability to differentiate closely related helminthes. Selective pressures on parasite populations (e.g. drugs, climate change, and wildlife host introductions) will continue to alter the composition of parasites on pasture-fed cattle and sheep. In addition, understanding the intestinal microenvironment will be critical in developing novel control strategies such as vaccines to control parasites. Researchers will also investigate the host response to the parasite to determine the role genetics of the host and parasite play in maintaining infestation and clearing the parasites. The application of classical and molecular tools to rapidly and reliably identify drug resistant parasites, the host's immune response, and the genomics of the host and parasite will be critical to managing and controlling parasitic diseases in the face of potential climate change and increased drug-resistance.

#### **Research Needs:**

Anti-helminthic resistance to drugs such as Ivermectin and Fenbendazole has been observed world-wide in nematodes of small ruminants, particularly in sheep-producing countries. Although the number of reports is low and resistance varies by region, there has been documentation of an increase in drug resistant

nematodes of cattle. Elucidation of the genetics of the immune response to parasites at both the host and parasite level will assist in the development of novel intervention strategies to reduce resistance to drugs by parasites.

**Anticipated Products:**

- Molecular-based techniques to rapidly speciate and quantify *Eimeria* oocysts in litter samples.
- Rapid tests to identify drug resistance markers in *Eimeria* field isolates.
- Recombinant vaccines that are safe and effective against heterologous field challenges with mass vaccination capability to prevent outbreaks of coccidiosis in poultry farms.
- Scientific information on cases of drug resistance related to parasite species; e.g., *Haemonchus contortus*, *H. placei*, *Cooperia punctata*, *C. oncophora*, *Ostertagia ostergii*, *Nematodirus helvetianus*, and *trichostrongyles*.
- Scientific information on the effect of genotypic and phenotypic differences of the host and parasite of drug resistance in sheep, cattle and goats.
- Molecular probes to better define parasite species in the field to enable tracking if their range changes due to climate change.
- Molecular markers of drug resistance based on mode of action and measure the allele frequency of parasite genes involved in drug resistance.
- Scientific information on patterns of gene flow in nematode populations to manage drug resistance in different production systems to reduce the impact of drug resistance on productivity.

**Potential Benefits:**

This research will provide a greater understanding of the extent and type of drug resistance in coccidia and nematodes of U.S. poultry, cattle and sheep. Improved molecular probes will be developed for speciating nematodes in the farm environment, and for identifying markers of drug resistance. A reduction in the incidence and effects of nematode infections in cattle and sheep is anticipated by allowing fact-based applications of appropriate anti-helminthic compounds.

**Problem Statement 6B: Hemoparasitic Diseases**

Hemoparasitic diseases result in significant export and production problems for the U.S. cattle and equine industries. Vector-borne diseases, which include the hemoparasites, was the 3<sup>rd</sup> priority for beef cattle stakeholders at the 2010 Animal Health workshop held in Baltimore, Maryland.

Anaplasmosis is one of the most prevalent arthropod-borne hemoparasitic disease and continues to constrain the production, movement, and utilization of cattle worldwide. Despite extensive losses impacting the major cattle producing regions of the world, immunization against the causative rickettsial pathogen, *Anaplasma marginale*, is impeded due to the lack of a safe and effective vaccine. Additionally, the lack of accurate diagnostic tools has restricted our ability to understand the epidemiology of infection. Current negotiations with Canada to export U.S. cattle are also limited due to

the lack of knowledge concerning risk factors such as vector competence within tick populations (especially in areas bordering Canada) and the contribution of tick efficiency in parasite transmission. Anaplasmosis is thought to be responsible for at least 50-100,000 cattle deaths per year in the United States with economic losses ranging from \$30-60 million. Sub-clinical losses including loss of weight and, at the clinical level, abortions and other complications, double or quadruple these estimates.

*Babesia* species are protozoan parasites of domestic and wild animals. They belong to the subclass commonly referred to as “piroplasms” due to the pear-like shaped merozoites that live as small intra-erythrocytic parasites. They commonly infect mammals, particularly cattle, sheep, goats, horses, pigs, dogs, cats, and occasionally man. *Babesia* has an unusual life cycle in that they include one-host ticks, belonging to the genus *Boophilus*. The parasites are passed to the eggs and hence to the larval stages, which can become infective after the adult tick dies, a process that is known as transovarian transmission.

*B. bovis* and *B. bigemina* are important causative agents of bovine babesiosis in tropical and subtropical regions of the world, while *Babesia divergens* is more common in temperate climates. Babesiosis was a significant problem in the southern United States until the 1940’s when it was controlled by eradication of the tick vectors through intensive acaricide dipping of cattle. However, the number of tick vectors present in the buffer zone along the Rio Grande, in Mexico, and in U.S. territories has been increasing as have the number of ticks found outside the quarantine zones. The increasing presence of these ticks poses a threat for reemergence into the United States, as evidenced by occasional outbreaks of babesiosis in the border region. There is a threat of reintroducing bovine babesiosis, a tick borne, hemoparasitic protozoal disease, into the United States from Mexico for the following reasons: 1) the USDA-APHIS surveillance program involves ticks only 2) at least a million cattle are moved north across the Mexican border each year, a percentage of which are *Babesia* carriers, 3) acaricide resistant ticks occur in northern Mexico and southern United States, 4) there is an increase in the number of wild ungulates along the border, and these and some cattle are not treated for ticks, and 5) there is no babesiacidal drug or vaccine approved for use in the United States.

The lack of a vaccine for control of babesiosis leaves U.S. cattle vulnerable to babesiosis upon reintroduction. It is estimated that the first year cost of controlling vector ticks alone, should they be introduced, into the United States, is over \$1.3 billion. *Babesia* also poses a public health threat. Species infective to humans are *Babesia bovis*, which can often be fatal, and *Babesia microti*, which is less pathogenic. In the United States, most of the hundreds of reported cases of babesiosis have been caused by *Babesia microti*, a parasite of small mammals transmitted by *Ixodes scapularis* (deer ticks); these ticks also transmit *Borrelia burgdorferi* (the cause of Lyme disease) and *Anaplasma (Ehrlichia) phagocytophila*.

Two products from research that would alleviate this threat are safe and effective vaccines for use in the United States (and elsewhere) and diagnostic assays capable of handling large numbers of samples for use in surveillance. *Babesia* vaccine development

requires the characterization of the protective immune mechanisms, the identification of protective antigens from the parasites, and the development of effective delivery systems. Babesial parasites have a complex life cycle including sexual stages in tick vectors and asexual reproduction during the erythrocytic stage in the mammalian host. Ideally, an effective anti-babesial vaccine will include parasite antigens of known function that will induce immune responses that prevent disease in the mammalian host and block transmission from tick vectors.

Equine piroplasmosis is another important tick-borne protozoal hemoparasitic disease that has tremendous impact on the movement of horses across international borders. Equine piroplasmosis has historically been exotic to the United States. A recent outbreak that appears to have originated in Texas has spread to at least 14 states to date. Piroplasmosis is difficult to diagnose, as it can cause variable and nonspecific clinical signs. The symptoms of this disease range from acute fever to anemia and jaundice, sudden death, or chronic weight loss, to poor exercise tolerance.

Equine piroplasmosis results from infection by the protozoa *Babesia caballi* or *B. equi* (phylum Apicomplexa), two organisms that may infect an animal concurrently. *B. caballi* and *B. equi* are transmitted by adult and nymphal ticks. *B. caballi* is spread by ticks in the genera *Dermacentor*, *Hyalomma*, and *Rhipicephalus*. *B. caballi* can be passed transovarially. *B. equi* is spread by ticks in the genera *Dermacentor*, *Hyalomma*, and *Rhipicephalus*. Recently, new tick reservoirs have been identified for this disease in the Western Hemisphere. *B. equi* does not appear to be passed transovarially. Equine piroplasmosis can also be spread by contaminated needles and syringes. Intrauterine infection of the foal is fairly common, particularly with *B. equi*. After recovery, horses may become carriers for long periods of time.

**Research Needs:**

Research is needed to discover vector-related contributions to the risk of these important hemoparasites in areas within the United States characterized as endemic and the discovery of parasite antigen structure associated with high transmission efficiency.

**Anticipated Products:**

- Scientific information on the transmission competence of vectors within the United States and trading partners (Canada and Mexico).
- Vaccines that prevent production losses from clinical disease and transmission (transfection technology is the center of our vaccine strategy for babesiosis).
- Scientific information on the effectiveness of current chemotherapeutics for *A. marginale*, *Babesia caballi*, and *B. equi* in clearing persistent infections.

**Potential Benefits:**

This research will provide data supporting and aiding decisions on import/export restrictions and novel vaccines to prevent clinical disease and block vector borne-transmission.

### **Component 6 Resources:**

The following ARS locations have research projects addressing the problem statements identified under Component 6:

- Pullman, WA
- Beltsville, MD

### **Component 7: Transmissible Spongiform Encephalopathies (TSEs)**

Transmissible spongiform encephalopathies (TSEs) include several fatal diseases of people and animals involving degeneration of the nervous system and brain function. TSEs are caused by agents known as prions, or what appear to be primarily infectious proteins that cause normal protein (cellular-prion protein PrP<sup>c</sup>) molecules to convert into an abnormally structured form (disease-prion protein PrP<sup>sc</sup>) that can include inducement of the formation of proteinaceous deposits and plaques in the brain. TSEs include Creutzfeldt-Jakob disease (CJD), the primary human prion disease; Scrapie of sheep and goats; Chronic Wasting Disease (CWD) of deer, elk, and moose; and Bovine Spongiform Encephalopathy (BSE), also called “mad cow,” which is the cause of variant CJD (vCJD) in people and the only TSE known to have crossed the species barrier from animals to people.

Our understanding of TSEs continues to evolve with ongoing research efforts. TSEs are progressive but long developing diseases. In humans, for example, incubation periods from the time of contact with an infectious prion may be decades long. Consequently, completion of research plans in natural hosts may require several years. Improvements have been made with the development of experimental rodent models to shorten the time required to obtain experimental results, but the relevance of any findings in mouse models remains uncertain unless confirmed and validated in natural hosts. In 2004, the Institute of Medicine of the *National Academies* published a report entitled: *Advancing Prion Science, Guidance for the National Prion Research Program*. Several federal agencies have directed resources to implement recommendations in the report, including HHS-NIH-, USDA-REE-ARS, HHS-FDA, HHS-CDC, DoD, and EPA. Although significant scientific advances have been made, the research conducted to date has yet to deliver many of the concrete solutions needed to safeguard people and animals from these devastating diseases. A critical concern is the potential for environmental, genetic, or iatrogenic events to lead to new variant TSEs that are infectious and zoonotic.

The White House Office of Science and Technology Policy (OSTP) Interagency Working Group (IWG) on Prion Science identified the following research priorities to maximize the impact of the National Prion Research Program:

- Identification of the nature and origin of prion agents
- Studies on the pathobiology of prion strains
- Research on the determinants of transmissibility and epidemiology
- Development of diagnostics, detection, and surveillance

These 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. Especially notable are the potential benefits to be derived from collaboration between animal health and human-biomedical research.

Stakeholders representing the sheep industries at our March 2010 Animal Health Workshop ranked research to eradicate Scrapie as their 2<sup>nd</sup> priority, and the goat industry listed it as their 6<sup>th</sup> priority.

### **Problem Statement 7A: Nature and Origin of Prion Agents**

Significant gaps remain in our understanding of the nature and origin of disease-causing prions. Proving especially problematic is that the normal prion protein is widely expressed, particularly on neurons in the brain, and is found on cell surfaces but its function is unclear. Moreover, the origin of BSE remains a mystery, although spontaneous conversion of bovine prion to the diseased form as occurs in human Creutzfeldt-Jakob disease is one researchable hypothesis. In addition, recent evidence indicates that some forms of BSE may be genetic in nature. Another enigma of TSEs in general is that different strains are found within the same animal species.

#### **Research Needs:**

The newly discovered strains of BSE and Scrapie, so-called “atypical” strains, have yet to be fully characterized. There are also fundamental differences between TSEs in different animal species. The factors responsible for host restrictions (species-barrier) are also not fully understood. An investment in this area of research is of paramount importance and will inform all other areas of prion research.

#### **Anticipated Products:**

Scientific information on:

- The physiological functions of normal prion proteins.
- The biophysical and biochemical properties of abnormal prion agents.
- Mechanisms of prion protein misfolding.
- The origin and prevalence of scrapie in goats.
- The origin and prevalence of atypical scrapie in sheep.
- The origin of atypical BSE and relationship to classical disease form.
- The basis for multiple TSE strains within a host species.

#### **Potential Benefits:**

This research will inform the field of prion science and is critical for advancing research programs in countermeasure discovery (see Problem Statement 7D below). Additional benefits will be derived from collaborations between animal health and biomedical research scientists resulting in animal disease models that will enhance our understanding of protein misfolding diseases, molecular neurology and genetics.

### **Problem Statement 7B: Pathobiology of Prion Strains**

Important gaps remain in our basic understanding of the pathobiology of animal prion diseases. One critical need is that of understanding the invasion routes of prions and resolving the variations seen in different animal species.

#### **Research Needs:**

It is widely assumed that the oral route of infection is important in the pathogenesis of naturally occurring TSEs of livestock and cervids; however, the mechanism of transmission of TSE agents from the initial site of entry to the central nervous system is not known. A notable feature of prion diseases is a lack of detectable immune responses and inflammation during the course of a prion infection, even though immune system cells may carry prions to target tissues. To date, research in animals suggests that prion accumulation may be largely influenced by the host species affected rather than the TSE involved. An investment in comparative pathology, which has not received much experimental attention, is needed to advance research programs in epidemiology and diagnostic discovery.

#### **Anticipated Products:**

Scientific information on:

- The manner in which prions enter the nervous system from peripheral sites of exposure such as a host's gastrointestinal tract, nasal mucosa, skin, and eyes.
- Mechanisms of prion spread within the nervous system.
- Mechanisms controlling disease incubation time.
- Mechanisms of neuropathogenesis.
- The molecular underpinnings of prion strains and species barriers.
- Prion distribution in goats with scrapie.
- Prion distribution in cattle with atypical BSE.
- Prion distribution in sheep with atypical scrapie and BSE.

#### **Potential Benefits:**

This research will inform the field of prion science of potential risks to human health associated with the newly emerging strains of TSE in various animal hosts and is critical for advancing research programs in epidemiology and diagnostic discovery (see Problem Statements 7C and 7D below).

### **Problem Statement 7C: Determinants of Transmissibility and Epidemiology**

In interspecies transmission studies of TSEs (e.g., Scrapie, CWD) into new hosts (e.g., cattle and rodent models that have proved useful in experimental protocols), scientists have demonstrated the extent of prion accumulation in tissues. There appears to be fundamental differences between hosts but also similarities within animal species, regardless of which TSE is affecting them.



**Research Needs:**

An essential research need is the development of infection models that represent real TSEs in real target hosts. The results of this research would add insight into human transmissibility and epidemiology.

**Anticipated Products:**

Scientific information on:

- Mechanisms by which abnormal prions are present in biological fluids.
- The infectious potential of prions from biological fluids.
- Infectivity time course and transmission.
- The potential for transmitting scrapie prions to cervids naturally (orally).
- The transmission routes of goat scrapie.
- The transmission routes of CWD.
- The transmission route of atypical scrapie and atypical BSE.
- The transmissibility of sheep scrapie to goats and vice versa, including the effects of age and genetic factors on transmissibility.
- The transmissibility of atypical BSE isolates to cattle.

**Potential Benefits:**

This research will directly impact the development of improved diagnostic tests (see Problem Statement 7D below), as well as surveillance programs and future measures to prevent the dissemination of TSEs in animal and human populations. The development of infection models in natural hosts will also build national capacity to implement research programs that target host-pathogen interactions and the discovery of countermeasures. Results from such studies could directly translate into a better understanding of the potential for the transmissibility of animal TSEs to humans.

**Problem Statement 7D: Diagnostics, Detection, and Surveillance**

Important gaps remain in our arsenal of diagnostic tools to detect TSEs. Current diagnostic tests were validated for use only on post-mortem samples; sensitive ante mortem tests have yet to be developed. Because there is no detectable immune response or inflammation during the course of TSE infection, direct tests are needed to confirm a diagnosis. At present, only highly-infected tissues, such as brain material or lymph tissue, are suitable for providing accurate diagnoses.

**Research Needs:**

Diagnostic approaches currently in use include techniques such as immunohistochemistry (IHC), Western blot, and enzyme-linked immunosorbent assays (ELISA). IHC is one of the original tests developed and is considered the gold standard, but it is more labor intensive and time consuming than the other two, whereas the Western blot and particularly ELISA tests are more efficient for the initial screening of large numbers of samples. Another method is the Conformation-Dependent Immunoassay (CDI), currently a research technique that claims to discriminate between normal prion and the abnormal prion on the basis

of its shape, but this has yet to be validated as a diagnostic test in animals. New technologies and methods have been described using protein misfolding cyclic amplification techniques (PMCA), similar in concept to gene/DNA amplification, which effectively increases the concentration of prions in normal or pathological conformations. There is a critical need to improve diagnostics methods for surveillance, including the discovery of an ante mortem test for early detection and implementation of intervention strategies.

**Anticipated Products:**

- TSE diagnostic test capable of detecting low levels of abnormal prions (i.e., key step to enable the development of an ante mortem test that can identify disease during the early stages of incubation).
- Validation of existing biopsy-based TSE tests in goats, deer, and elk.
- Rapid biochemical methods for strain typing.
- Validated murine models for strain typing.
- Improved diagnostics for TSEs in bodily fluids, including blood in host species where this might be possible.
- Technologies to distinguish infectious prions from normal cellular prion proteins.
- Determination of the prevalence of proteinase K sensitive prion in the various TSEs and potential of this form to cause disparate results between IHC, WB, and ELISA tests.

**Potential Benefits:**

The discovery of an ante mortem diagnostic test would enable the medical community to test and discover effective treatments in people. Importantly, new and improved diagnostic platforms and an ante mortem diagnostic test for susceptible livestock will enable early detection and the implementation of effective surveillance programs, a critical step that will allow the deployment of disease prevention measures.

**Component 7 Resources:**

The following ARS locations have research projects addressing the problem statements identified under Component 7:

- Ames, IA
- Albany, CA
- Ames, IA
- Pullman, WA