Action Plan
National Program 103
Animal Health

Program Summary:

Program Direction

Vision Statement
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.

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

Process for Establishing this NP Plan
An independent retrospective peer assessment of National Program (NP) 103 past performances was conducted in October 2004. A national electronic survey was also conducted in 2005 to obtain input on past performance and future research priorities. The reports (hyperlink) were presented at a Stakeholder workshop on September 19-20, 2005 in Kansas City MO. Research priorities developed with stakeholder input for the next cycle (hyperlink) served as the basis for an Action Plan for research that is relevant to the needs of U.S. agriculture.

Program Components
1. Biodefense research
2. Animal genomics and immunology
3. Zoonotic diseases
4. Respiratory diseases
5. Reproductive and neonatal diseases
6. Enteric diseases
7. Parasitic diseases
8. Transmissible spongiform encephalopathies

Action Plan

Action Plan: National Program 103 – Animal Health

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

Strategic Objectives:

1. Establish ARS laboratories into a fluid, highly effective research network, to maximize use of core competencies and resources

2. Access to specialized high containment facilities to study zoonotic and emerging diseases

3. Develop an integrated animal and microbial genomics research program

4. Establish centers of excellence in animal immunology

5. Launch a biotherapeutic discovery program providing alternatives to animal drugs

6. Build a technology-driven vaccine and diagnostic discovery research program

7. Develop core competencies in field epidemiology and predictive biology

8. Develop internationally recognized 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

Program targets:

- Find new solutions to prevent economic losses from domestic and foreign animal diseases in agriculture species.
- Develop methods to help producers adjust to changing farming practices that will allow consumer driven issues to be accommodated without compromising financial viability.
- Provide information that will allow the establishment of on-farm practices to maximize biosecurity from naturally or intentionally introduced pathogens that will increase food security, farm productivity, and enhance trade and exports.
- Establish methods to be able to detect, analyze and respond to new and emerging agriculture pathogens.
- Find solutions to maintaining a barrier to pathogens at the domestic-wildlife interface.
- Establish new detection technologies that will allow better tracking and control of animal pathogens.
- Build an integrated research program 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.

Achieving results in veterinary medical research, which provide useful information for problem-solving, often demands an integrated approach in which the experimental design may range from knowledge development at the molecular level to clinical trials that will lead to the development of countermeasures for preventing and controlling a disease outbreak in the field. This NP provides the means for the integration of research. For this purpose, select NP 103 projects will be aligned under "Major Initiatives." Each major initiative will be outlined as problem statements under the research components of the action plan. Major initiatives will draw upon relevant expertise within NP 103, coordinating and integrating that expertise to develop a specific useful application of the knowledge. Major initiative projects may also attract federal, university, industry and international partners. Objectives of major initiative projects will be consistent with those of their base projects. If successful, therefore, they will enhance, rather than detract from, the impact of those base projects on their assigned topics. Because a significant number of projects in the animal health research portfolio focuses on the discovery of novel technologies, intellectual property strategies will be addressed in project plans to facilitate technology transfers and the investment by the private sector in the development of these technologies.

Relationship of This National Program to the ARS Strategic Plan: Outputs of NP 103 research support the "Actionable Strategies" associated with the performance measures shown below from the *ARS Strategic Plan for 2003-2008,* Objective 3.2: *Develop and Deliver Science-Based Information and Technologies to Reduce the Number and Severity of Agricultural Pest, Insect, Weed, and Disease Outbreaks.*

Performance Measure 3.2.1: Provide scientific information to protect animals from pests, infectious diseases, and other disease-causing entities that affect animal and human health. **Target:** Increase the delivery of dependable high quality scientific information to customers, stakeholders, and partners. New discoveries and technologies will be effectively communicated to improve the management of diseases that affect the livestock and poultry industries, and which may affect public health. Effective communication will be achieved by publishing in highly regarded scientific journals and trade publications and on the internet and through presentations at industry meetings.

Performance Measure 3.2.2: Identify, develop, and release to the U.S. agricultural community genetic markers, genetic lines, breeds, or Germplasm that result in food animals with improved (either through traditional breeding or biotechnology) pest- and disease-resistance traits. **Target:** Release new and improved genetic lines, breeds, and/or Germplasm of food animals that exhibit enhanced pest- and disease-resistance traits.

Performance Measure 3.2.3: Develop and transfer tools to the agricultural community, commercial partners, and Federal agencies to control or eradicate domestic and exotic diseases that affect animal and human health. **Target:** Develop diagnostic and preventative tools to control and/or eradicate domestic diseases that affect production, trade, and public health. Provide action agencies with data to support risk analyses to assess the impact of domestic and exotic diseases and develop control and eradication strategies.

Major Initiatives

Component 1: Biodefense Research
Problem Statement 1A: Foreign Animal Diseases
Problem Statement 1B: Emerging Diseases

Component 2: Animal Genomics and Immunology
Problem Statement 2A: Mastitis
Problem Statement 2B: Avian Tumor Viruses
Problem Statement 2C: Mucosal Diseases of Livestock and Poultry

Component 3: Prevention and Control of Zoonotic Diseases
Problem Statement 3A: Brucellosis
Problem Statement 3B: Leptospirosis
Problem Statement 3C: Tuberculosis

Component 4: Prevention and Control of Respiratory Diseases
Problem Statement 4A: Ruminant Respiratory Diseases
Problem Statement 4B: Porcine Respiratory Diseases
Problem Statement 4C: Poultry Respiratory Diseases

Component 5: Prevention and Control of Reproductive and Neonatal Diseases
Problem Statement 5A: Bovine Viral Diarrhea (BVD)
Problem Statement 5B: Neosporosis
Problem Statement 5C: Reproductive Health of the Dairy Cow

Component 6: Prevention and Control of Enteric Diseases
Problem Statement 6A: Johne's Disease
Problem Statement 6B: Enteric Diseases of Poultry

Component 7: Prevention and Control of Parasitic Diseases
Problem Statement 7A: Drug Resistant Gastrointestinal (GI) Parasitic Diseases
Problem Statement 7B: Hemoparasitic Diseases

Component 8: Prevention and Control of Transmissible Spongiform Encephalopathies
Problem Statement 9A: Scrapie
Problem Statement 9B: Chronic Wasting Disease (CWD)
Problem Statement 9C: Bovine Spongiform Encephalopathy (BSE)

Component 1: Biodefense Research

Good animal health 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, ease of control and ability to eradicate. 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 counter agro terrorism program 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 and 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 researchers and facilities in other countries are therefore essential in the preparedness for an animal disease invasion. The program must include research on how diseases agents survive 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 to reduce the chance of organism introduction. In order to respond to a disease incursion, research must provide tools for accurate and continuous surveillance programs. To counter an animal disease, research programs must include ways to manipulate the animal's immunological resistance to infection and ways to increase animal disease immunological resistance through genetic selection. This increased general resistance to infection is the best way to minimize economic loss and disease spread. Increased research on general treatments that target common pathways of agent-host infection will have the most benefit since specific vaccines for each disease cannot be created or stockpiled. To ensure producers are returned to full production as soon as possible, research much provide means to prove that animals are free of the disease that are accepted by the international community.

Problem Statement 1A: Foreign Animal Diseases

Foreign animal diseases represent a major threat to U.S agriculture. Stakeholders at the September 2005 Animal Health Planning Workshop representing the beef industry ranked biodefense research on foreign animal diseases as their 1st priority; the poultry layer industry their 1st priority; the poultry broiler industry their 2nd priority; the dairy industry their 5th priority; and the swine industry their 5th priority. 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, Avian Influenza, Rift Valley Fever, Classical Swine Fever, Exotic Newcastle disease, Vesicular stomatitis, Exotic bluetongue, and Monkeypox virus. Additional diseases that impact trade are addressed under Component 7, Babesiosis, 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 U.S and its territories that has a potential significant health or economic impact. Foreign animal diseases are considered a threat to the U.S when they significantly affect human health or animal production and 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 a FAD must be rapidly controlled.

In the U.S, 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 U.S will be 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. Our understanding of pathogenesis, transmission, and immune response is insufficient to rapidly control and eradicate foreign animal diseases. 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 U.S more effective prevention and control tools such as identifying suitable control strategies compatible with short time and cost of recovery from disease outbreaks (DIVA compatible) need to be developed. There is a need for development of vaccines and biotherapeutics suitable for strategic stockpiles, integrated methods of disease control including vector control and animal management, which all lead to a better capability to regain country disease-free status and retain economic sustainability.

Outputs:
Better anticipation of introduction of foreign animal diseases

Capability to advise regulatory officials on scientific procedures for the prevention of introduction of FADs
Better capability to produce effective products to control and eliminate foreign animal diseases
Real-time detection of agents in a wide range of farm matrices
Searchable databases of genome and proteome information for major known FAD agents
Improved ability to predict or anticipate emergence or introduction FAD agents
Discovery of effective candidate biotherapeutics
Discovery of effective candidate vaccines that allow differentiation of infected animals from vaccinated animals (DIVA).
Viable integrated vector control strategies that minimize losses

Impact:
This research will lead to better methods for prevention and control of high consequence diseases.

Resources:
Eight (8) ARS CRIS projects are coded to National Program 103 address the research problems identified under Component 1A. Fourteen (14) ARS scientists who are assigned to these projects include:

Athens Georgia	Suarez, David; Swayne, David; King Jack; Spackman Erica
Laramie Wyoming	Wilson, William; Mecham Jim; Stith Charlie; Drolet Barb
Orient Point New York	Rodriguez, Luis; Grubman, Marvin; Borca Manuel; Baxt Barry; Reider Elizabeth, Gregg Doug

Problem Statement 1B: Emerging Diseases
Several new emerging animal disease issues appear every year. 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—all these factors 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 U.S, 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.

Research needs:
Capability to rapidly identify, characterize, control and eradicate a new animal pathogen of high economic consequence is not well developed. There is a need to isolate, identify, and characterize pathogen(s) associated with new disease complexes of unknown etiologies. Scientists need to conduct challenge studies to fulfill Koch's postulate and determine the pathogenesis of monovalent and multivalent infections. Once a new agent is isolated there is a need to sequence partial or complete microbial genomes to identify unique sequences for diagnostic discovery and molecular epidemiology research. Research need to identify mechanisms of disease, disease transmission, and host range specificity to determine the prevalence and emerging potential of new diseases. Ultimately good research will lead to predictors of disease emergence and disease outbreaks.

Outputs:
Identification of new pathogens associated with emerging diseases
Predictors of emerging livestock diseases
Establishment of methods to rapidly detect and characterize the etiology of new and emerging diseases.
Development of predictors of disease outbreaks.
Establishment of early warning systems for emerging diseases.
Tools and expertise to prevent emerging diseases and rapidly implement countermeasures to respond to new disease outbreaks.

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

Resources:
Two (2) ARS CRIS projects are coded to National Program 103 address the research problems identified under Component 1B. Four (4) ARS scientists who are assigned to these projects include:

Ames Iowa	Kehrli, Marcus; Lager Kelly
Athens Georgia	Suarez, David; Swayne, David

<u>Component 2: Animal Genomics and Immunology</u>
Genetic disease susceptibility is one of three core components of the 2003-2008 ARS Strategic Plan for animal health research. A critical goal is the development of genomic-based assays to select for disease susceptibility traits, in targeted animal populations, and defined animal production systems. With the availability of the human genome sequence there has occurred a fundamental paradigm shift in biomedical research. Characterization of the genome and definition of the variation found among individuals has led to the discovery of the genetic basis of susceptibility to complex disease states. Similarly, the application of animal genomics has the potential to revolutionize the speed and scope of problem-solving in animal health. Animal genomics promise new powerful approaches

to address key questions in basic animal biology that will lead to improved countermeasures to prevent and control diseases.

The last fifty years was characterized by tremendous strides in improving animal breeds based on quantitative genetics and the selective breeding of farm animals. Most of the successes have been achieved through selection using traditional quantitative genetics tools, but quantitative trait loci (QTL) mapped for easily measurable production traits (e.g., milk yield) have recently had impact on selection programs. However, models describing the genetic control of complex traits such as disease resistance have been unreliable when applied to outbred populations under field conditions. Importantly, the number of genes, the extent of their effect on disease susceptibility, and the interactions between them remain unknown. Recent efforts to sequence the chicken and bovine genomes provide the means to shift from traditional quantitative genetics, to genomic approaches that promote the understanding of genetics and gene function. Availability of nearly limitless sources of putative genetic markers in specific genomic regions will facilitate fine-mapping of QTL and identification of positional candidate genes. Marker-assisted selection using whole genome prediction may become feasible. We now have molecular tools that will allow the expression of thousands of genes to be studied simultaneously. The availability of entire animal genome sequences and knowledge of the extent of variations within agricultural animal populations will enable studies in transcriptomics, proteomics, and metabolomics and genome-wide expression analysis microarrays or RNAi gene knock-down. The application of animal genomics research in animal health offers unparallel opportunities to fulfill our mission.

The ARS animal health genomics research program will use available resources to focus on three strategic areas: 1) understanding the genetic and biological determinants of disease susceptibility, including the development of genomics tools to identify and select for disease susceptibility traits; 2) understanding mechanisms of protective immunity, pathogen immune evasion, and the role of immunogenetics and genomic variances in innate and adaptive immune responses; and 3) the development of highly effective genomics-based diagnostics, vaccines, and biotherapeutics to prevent and control priority diseases in target farm animal populations.

Stakeholders at our September 2005 Animal Health Planning Workshop ranked cross-cutting issues, such as animal genomics to improve animal health their 2nd priority. Representatives of the dairy industry ranked periparturient diseases their 2nd priority, with mastitis and immunosuppression the most important diseases affecting the dairy cow. The poultry broiler industry identified production alternatives (e.g., antibiotic growth promoters) to control diseases such as Necrotic Enteritis as their 1st priority and enteric diseases such as coccidiosis their 4th priority. The sheep industry identified ovine progressive pneumonia virus (OPPV) and immunogenetics as their 3rd priority.

Problem Statement 2A: 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 can result in the death of the animal. 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, greater than half of economic losses are due to sub-clinical mastitis. Sub-clinical mastitis is often associated with an elevation in the number of somatic cells in the bulk tank of the dairy farm. The National Mastitis Council reports calculated herd losses at 6% for bulk tank somatic cell count (BTSCC) at 500,000 cells/ml, and 18% for BTSCC at 1,000,000 cells/ml, when compared to herds at 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 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. 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 mastitis pathogens that cause clinical mastitis in cattle. Research has shown that intramammary antibiotic treatment may not be cost effective and may be possibly detrimental to the cow's health. Importantly, organic producers have almost no options available to them for the prevention and treatment of mastitis.

Research Needs:

The goal of the ARS mastitis research program is to develop genomic- and immunologic-based strategies to prevent and control bovine mastitis. Apply a functional genomics approach to understand variations in gene expression in bovine neutrophils in response to Gram-negative versus Gram- positive bacterial challenges of the mammary gland. A new understanding of gene interactions involved in immune cell activation, migration, and host responses will be used to discover effective vaccines to prevent mastitis. New tools based on protective host proteins will be developed to modulate the dairy cow's innate immune response to treat infections and/or increase host clearance of bacteria. Discover new approaches to treat severe mastitis by limiting the inflammatory response, reducing death of mammary secretory cells and promoting the replacement of damaged cells. A genomics approach will be used to increase the levels of naturally expressed anti-bacterial peptides in the mammary gland, either through administration of recombinant derivatives, genetic selection, and/or transgenics.

Outputs:

Identify genetic variations associated with difference in immune cell activation, migration, and host responses to Gram-positive versus Gram-negative bacteria.

Effective vaccines to prevent mastitis.

New biotherapeutic platforms based on protective host proteins to induce the cow’s innate immune response.

Therapeutics to reduce cell damage during mastitis.

Markers for mammary stem cells and methods for their regulation.
New biotherapeutic platforms based on targeted expression of host-derived inflammatory products with receptor antagonists and/or siRNA molecules.
Innovative approaches using naturally expressed anti-bacterial peptides to increase resistance to prevalent pathogens.
New management and nutritional schemes to prevent metabolic stresses contributing to immunosuppressive states in the dairy cow.

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

Resources:
Two (2) ARS CRIS projects are coded to National Program 103 address the research problems identified under Component 2A. Six (6) ARS scientists who are assigned to these projects include:

Beltsville MD Bannerman, Douglas; Capuco, Anthony; Paape, Max

Ames IA Goff, Jesse; Lippolis, John; Reinhardt, Timothy

Problem Statement 2B: Avian Tumor Viruses
Avian tumor viruses are endemic in the U.S. poultry industry 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 insure breeder flocks remain virus free. These control measures conservatively cost the U.S. poultry industry in excess of 200 million for vaccination and 20 million in diagnostic tests annually. More importantly, there is a fifty-year history of these viruses evolving to render the latest control measures ineffective.

Avian tumor 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, a new recombinant virus consisting part of ALV-A and part 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 industries

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, the primary economic concerns of REV infection are as a barrier to export of 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 (The first vaccine ever developed to prevent a cancer). However, current vaccines do not block viral infection and spread. It is speculated that vaccine selection pressures have resulted in new highly virulent viral strains that have been reported to cause greater than 50 percent mortality in certain flocks. Continued reports of periodic MD outbreaks in vaccinated flocks all point to the need for new strategies to control this re-emerging viral disease. In the absence of control measures, MD is capable of causing devastating losses in commercial layer and broiler flocks. As a disease occurring worldwide, with increasing reports of vaccination breaks and emergence of more virulent pathotypes, MD poses severe threats to the poultry industry and developing strategies for its control remains one of the great challenges today.

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.

Outputs:
Basic research information to understand how genetic variations influence the immune response to Marek's disease infection.
Basic research information to understand 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.

Identification of viral genes responsible for pathogenesis and identification of predictors of virulence shifts.

Elucidation of viral genes associated with immune evasion mechanisms.

Characterization of biological pathways that lead to the development of Marek's disease.
Characterization of vaccine-induced determinants of protective immunity.

A safe and highly effective vaccine with mass vaccination capability that conveys protection against emerging Marek's disease viral strains in defined host animal genotypes.

Impact:
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.

Resources:
Two (2) ARS CRIS project coded to National Program 103 address the research problems identified under Component 2B. Seven (7) ARS scientists who are assigned to these projects include:

East Lansing MI	Cheng, Hans; Fadly, Aly; Heidari, Mohammad; Hunt, Henry; Lee, Lucy; Silva, Robert; Zhang, Huanmin
Athens GA	Spatz, Steven

Problem Statement 2C: Mucosal Diseases of Livestock and Poultry
Mucosal surfaces are extremely complex portals to the gastrointestinal and respiratory 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. 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. Mucosal surfaces also represent the main sites of interaction between the host and environmental organisms, so called commensal organisms that provide important benefits to the host such as nutrients and abundant immunostimulatory molecules.

Mucosal 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 mucosal diseases of livestock and poultry. The research will focus on the following priority diseases: Necrotic enteritis of poultry (NE); avian coccidiosis; avian

influenza; porcine reproductive and respiratory disease syndrome (PRRS); Enzootic pneumonia (*Mycoplasma hyopneumoni*ae); gastrointestinal nematodes; Ovine progressive pneumoniae (OPP); and Parainfluenza 3.

Research Needs:

Mucosal pathogens include some of the most important pathogens affecting intensive production of livestock and poultry. Many of the available tools for preventing and controlling these diseases are ineffective. 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 mucosal pathogens in the field. Because many of these diseases are complex entities involving multiple pathogens and multiple disease resistance pathways, genomic approaches are required to address issues of pleotrophism and gene epistasis. High throughput genomic approaches will be interfaced 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 mucosal 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 3, 4, 6, and 7).

Outputs:

Discovery of genes that confer susceptibility to mucosal diseases of cattle, pigs, and poultry.

Identification of genomic regions or specific structural variations that are associated with differences in pest- and disease-resistance traits.

Improved methods of predicting genetic merit based on dense SNP-based marker data.

Development of enabling tools (immunological reagents, SNP markers and SNP Haplotypes) to propel our understanding of host responses to mucosal diseases.

Identification of the genes mediating resistance from previously determined quantitative trait loci (QTL) associated with susceptibility to mucosal diseases of livestock and poultry.

Differences in host immunological and physiological responses to mucosal diseases will be associated with genetic markers and then fine-mapped to specific genes.

Gene expression and genetic variations associated with protective mucosal immune responses will be identified and characterized.

Biological determinants of innate and adaptive protective immunity will be identified and characterized.

Comparative genetic maps to identify areas of similarity (synteny) between the bovine, swine, avian, humans, and mice genome.

Identification of markers for the detection of underlying mucosal disease infections.

Highly effective diagnostics, vaccines, and biotherapeutics designed to prevent and control mucosal diseases in targeted animal populations.

Impact:

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.

Resources:
Five (5) ARS CRIS projects coded to National Program 103 address the research problems identified under Component 2C. Thirteen (13) ARS scientists who are assigned to these projects include:

Beltsville MD	Gasbarre, Lou; Lillehoj, Hyun; Lunney, Joan; Miska, Katarzena; Urban, Joseph
Clay Center NE	Clawson, Michael; Heaton, Michael; Laegreid, William; Miller, Laura
Pullman WA	Herrmann, Lynn; Kappmeyer, Lowell; Knowles, Don; White, Stephen

Component 3: Prevention and Control of Zoonotic Diseases
Zoonotic diseases represent one of the leading causes of illness and death in people. By definition, zoonotic diseases encompass all infectious diseases that are transmitted from animals to man. Zoonotic diseases have a negative impact on commerce, travel, and economies worldwide. In developing countries, zoonotic diseases stand out as the most prevalent and important threat to public health. In industrialized nations, zoonotic diseases 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, the USDA has invested significant resources in attempts to eradicate endemic zoonoses from livestock populations (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 the source and protect our Nation from these important public health threats.

The ARS zoonotic diseases research program will focus on brucellosis, leptospirosis, and tuberculosis with the strategic goal of developing countermeasures to prevent disease transmission in domestic livestock and wildlife reservoir hosts. Zoonotic diseases that pose a significant threat to the Nation (e.g., avian influenza, Rift Valley fever) and are exotic to the U.S are addressed under Component 1: Biodefense Research. Additional zoonotic diseases are also addressed under Component 7B (Babesiosis) and Component 8 (BSE).

Stakeholders at the September 2005 Animal Health Program Planning Workshop representing the swine industry ranked zoonoses as their 2nd priority; the wildlife industries as their 2nd priority; the dairy industry as their 3rd priority; and the beef industry their 4th priority.

Problem Statement 3A: Brucellosis

Brucellosis is one of the most important 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 and swine, which can result in dire economic losses once a herd becomes infected. Brucellosis has been subject to an intensive eradication campaign in the U.S for the last fifty years. Certain *Brucella* species tend to have a predilection for certain animal species (e.g., *B. abortus*-cattle, *B. suis*-pigs, *B. melitensis*-sheep) but many can infect all mammals with varying degrees of virulence. Wildlife species can be an important source of infection; for example, the much publicized bison in Yellowstone Park, but also elk and feral swine. Most importantly, brucellosis has recently been classified by the U.S biodefense community as a potential agent for bioterrorism.

Tremendous strides have been made in eradicating brucellosis from U.S cattle and swine. Brucellosis annual losses from 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. However, 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. 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.

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

Research Needs:
Genomic analyses of *Brucella species* to identify unique sequences. Immunologic responses in wildlife and domestic livestock to wild type *Brucella species* and experimental vaccines will be characterized. Improved diagnostic and vaccine countermeasures specifically designed for the control and eradication of brucellosis in wildlife will be discovered.

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

Impact:
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.

Resources:
One (1) ARS CRIS project is coded to National Program 103 to address the research problems identified under Component 6A. Four (4) ARS scientists who are assigned to this project include:

Ames IA Bricker, Betsy; Halling, Shirley; Olsen, Steven; Stoffregen, William

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. Case fatality rates can be 20 percent or higher. *Leptospira* includes over 200 serovars that can infect most animal species. Importantly, *Leptospira* can be acquired directly from the environment, which places it in the category of diseases that cannot be eradicated. It can result in significant economic impact due to the international trade of animals and semen. Economic losses are also caused by the cost for treatment and control and by 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. The two main reasons are that 1) Leptospirosis is very difficult to diagnose and 2) most endemic countries (including the U.S) 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 vulnerable to interpreter variations. Most commercial tests cannot distinguish between serovars. New diagnostic technologies are therefore needed and are a priority. Recent global epidemiological surveys indicate that the most prevalent serovar is Icterohaemorrhagiae, followed by Pomona, Sejroe, Australis, Autumnalis, Grippotyphosa, and Canicola . Rodents, rats and mice composed half of the reported source of infection. However, almost 40 percent were from farm animals and companion animals. 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. Whole herd vaccination is the primary method of control. 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.

Outputs:

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

Impact:
Functional genomics analysis of *Leptospira* strains will enable the identification of virulence determinants, vaccine discovery research, and new diagnostic platforms for classification of field strains.
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.

Resources:
One (1) ARS CRIS project is coded to National Program 103 to address the research problems identified under Component 6A. Four (4) ARS scientists who are assigned to this project include:

Ames IA Alt, David; Elliott, Margaret; Zuerner, Richard

Problem Statement 3C: Tuberculosis
Bovine tuberculosis eradication efforts were initiated in the United States in 1917. The crux of the eradication program is based on abattoir inspections, testing, and depopulation of infected herds. These efforts have been largely successful. The reactor rate in cattle has been reduced from about 5 percent to currently less than 0.02 percent. Consequently, the incidence of human tuberculosis caused by *Mycobacterium bovis* has also decreased significantly. It is estimated that between 1917 and 1962 the USDA tuberculosis eradication campaign cost $3 billion in 2003 dollars. During this same period it is estimated that the annual savings were 12 times the cost in decreased carcass condemnation and improved animal productivity. 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, as the incidence of tuberculosis in the U.S declines, Federal and State control programs are facing new challenges. First, there has been a resurgence of bovine tuberculosis in recent years, both in our domestic cattle herds and wildlife. This resurgence is due to several factors: the importation of *Mycobacterium bovis* infected cattle from Mexico; infections in captive deer and elk herds; the presence of tuberculosis in zoo and wildlife species maintained for exhibition; and most recently, the emergence

of the first free-ranging wildlife reservoir (i.e., white-tailed deer) in the U.S. The detection of tuberculous cattle and wildlife has serious economic consequences, primarily due to restrictions imposed by regulatory officials on the interstate and international shipment of livestock. As a result, the Animal and Plant Health Inspection Service (APHIS)-USDA 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.

Research Needs:
The identification of microbial immunogens critical for the induction of protective immunity is needed. 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.

Outputs:
Identification of 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
Discover improved sensitive and specific diagnostic platforms amenable to the rapid screening of large cattle herds.
Discover diagnostic platforms to differentiate infected versus vaccinated animals.
Discover effective vaccine platforms to prevent and control *Mycobacterium bovis* in cattle and relevant wildlife reservoir hosts.

Impact:
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.

Resources:
One (1) ARS CRIS project is coded to National Program 103 to address the research problems identified under Component 3C. Three (3) ARS scientists who are assigned to this project include:

Ames IA Palmer, Mitchell; Waters, Wade; One Vacancy

<u>Component 4: Prevention and Control of Respiratory Diseases</u>
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 today, respiratory lesions are still prevalent at slaughter and their impact on weight gain and carcass quality is significant. Respiratory diseases continue to be 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 September 2005 Animal Health Workshop representing the beef industry ranked respiratory diseases as their 2nd priority; the poultry broiler industry their 3rd priority; and the swine industry their 4th priority. Stakeholders from the swine industry ranked complex disease interactions, including respiratory diseases, as their 4th priority, but they ranked porcine reproductive and respiratory syndrome virus and re-emerging diseases, such as swine influenza, as their 1st and 3rd 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: Ruminant Respiratory Diseases

Respiratory disease of beef cattle is the most costly disease facing producers today, estimated at $3 billion to the U.S cattle economy annually, and is among the most costly diseases for dairy, sheep and goat producers. The U.S 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 U.S, however, produces an exceptional challenge to efforts directed at disease control. Movement of cattle from cow-calf operations to stockers to feedyards 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 controversial tools used to raise cattle and will continue to raise safety standards. Varieties of vaccines 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 much less used 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 at our September 2005 Animal Health Workshop representing the beef industry characterized bovine respiratory disease as their 2nd priority. Specific etiologic agents listed were BVDV (see **Component 5A**), *Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somni* (previously *Haemophilus somnus*), and emerging viral co-infections. The sheep industry ranked respiratory diseases such as Malignant Catarrhal Fever (MCF) as their 5th priority.

Research Needs:
Research will be conducted to define mechanisms of disease transmission of respiratory pathogens in relevant beef 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. Develop improved diagnostic capabilities that will enable rapid diagnosis 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.

Outputs:
Define determinants of virulence and characterize mechanisms of infection.
Define pathogen interactions that lead to polymicrobial infections and respiratory disease complexes.
Characterize mechanisms of immune evasion and protective immunity.
Develop drug and vaccine delivery systems that target the ruminant respiratory tract.
Discover and evaluate alternatives to antibiotics for preventing and treating respiratory diseases.
Discover diagnostic platforms that can be used to develop on-site tests.
Discover highly effective vaccines that induce targeted immune responses to prevent colonization of the respiratory tract and prevent shedding and disease transmission.

Impact:

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.

Resources:
Two (2) ARS CRIS project coded to National Program 103 addresses the research problems identified under Component 4A. Five (5) ARS scientists who are assigned to these projects include:

Ames, IA Briggs, Robert; Lehmhuhl, Howard; Sacco, Randy; Tatum, Fred;

Pullman WA Knowles, Donald; Li, Hong; Taus, Naomi

Problem Statement 4B: Porcine Respiratory Diseases
The U.S. swine industry is the 3rd 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 U.S. is also the 2nd 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. Because of this, respiratory disease in pigs is the most important health concern for swine producers today. Respiratory disease in swine, as with other species, is generally considered a multifactorial problem caused by a combination of viral and bacterial infectious agents, as well as adverse environmental conditions. The National Pork Board has consistently listed the porcine respiratory disease complex (PRDC) as a top research priority. 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 multifactorial 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 September 2005 Animal Health Program Planning Workshop in Kansas City ranked PRRSV (also see **Component 2C**) as their 1st priority, preventative health management (innate immunity and alternatives to growth promotants and antibiotics) as their 2nd priority, re-emerging diseases such as SIV and PCV2 as their 3rd priority, and the porcine respiratory disease complex (PRDC) their 4th priority.

Research Needs:
The pathogenesis and polymicrobial interactions of respiratory pathogens will be investigated. Research will be conducted to define mechanisms of disease of swine respiratory pathogens 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.

Outputs:
Define pathogen interactions that lead to polymicrobial infections and respiratory disease complexes in swine.
Characterize the changes in gene expression underlying porcine cellular responses to infection with respiratory pathogens.
Characterize global changes in gene expression of porcine bacterial pathogens in response to respiratory infection.
Define determinants of virulence and characterize mechanisms of infection.
Genomic and proteomic analysis of respiratory pathogen to determine changes in gene expression during the infection process.
Identification of microbial genetic variations associated with differences in virulence and disease transmission.
Characterize mechanisms of immune evasion and protective immunity.
Discover highly effective vaccines that induce targeted immune responses to prevent colonization of the respiratory tract and prevent shedding and disease transmission.

Impact:
The overall impact of the research will be improved control and prevention of endemic respiratory diseases that will benefit the swine industry. 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.

Resources:

Two (2) ARS CRIS projects coded to National Program 103 address the research problems identified under Component 4B. Nine (9) ARS scientists who are assigned to these projects include:

Ames, IA	Brockmeier, Susan L; Cheung, Andrew; Kehrli, Jr., Marcus, E; Lager, Kelly M; Nicholson, Tracy L; Register, Karen B; Richt, Juergen; Sacco, Randy E; Wesley, Ronald D.

Problem Statement 4C: Poultry Respiratory Diseases

The U.S poultry industry is the most productive worldwide with 15 million metric tons of broiler meat produced in 2004. The industry is fully integrated and supports the intensive production of 8 billion birds annually. However, endemic respiratory diseases continue to decrease the profitability of commercial poultry production. As we enter the 21st 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 dependant 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. The USAHA-2004 Poultry Report 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 influenzae, and infectious bronchitis virus; whereas the turkey industry identified avian pneumovirus, *Escherichia coli (colibacillosis), Ornithobacterium rhinotracheale* (ORT) , *Bordetella avium* (turkey coryza), and *Pasteurella multocida* (fowl cholera) as problematic.

Stakeholders at our September 2005 Animal Health Workshop representing the poultry industry characterized the following respiratory pathogens as priority diseases: infectious bronchitis, infectious laryngotracheitis, turkey rhinotracheitis (TRT) and associated swollen head syndrome (SHS) of chickens (avian pneumovirus), colibacillosis

(*Escherichia coli*), fowl chorea (*Pasteurella multocida*), turkey coryza (*Bordetella avium*), *Ornithobacterium rhinotracheale* (ORT) of turkeys, and Mycoplasmosis (*Mycoplasma gallisepticum* and *Mycoplasma synoviae*). The 2005 ARS National Stakeholder (Electronic) Survey ranked infectious bronchitis and infectious laryngotracheitis as priority endemic respiratory diseases.

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. 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.

Outputs:
Define the characteristics of aerosol spread for priority respiratory pathogens in relevant poultry production systems.
Define determinants of virulence and characterize mechanisms of infection.
Define pathogen interactions that lead to polymicrobial infections and respiratory disease complexes.
Characterize mechanisms of immune evasion and protective immunity.
Develop drug and vaccine delivery systems that target the avian respiratory tract.
Discover and evaluate alternatives to antibiotics for preventing and treating respiratory diseases.
Discover differential diagnostics platforms that can be used to develop flock-side tests
Discover highly effective vaccines that induce targeted immune responses to prevent colonization of the respiratory tract and prevent shedding and disease transmission.

Impact:
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.

Resources:
Four (4) ARS CRIS projects coded to National Program 103 address the research problems identified under Component 4C. Fifteen (15) ARS scientists who are assigned to these projects include:

Ames, IA	Briggs, Robert E; Register, Karen B; Tabatabai, Louisa B; Tatum, Fred M
Athens, GA	Kapczynski, Darrell; Yu, Qingzhong; One Vacancy
Fayetteville, AR	Huff, William E.; Donoghue, Ann M.; Huff, Geraldine R.; and Rath, Narayan C.
Mississippi State, MS	Branton, Scott; Evans, Jeff; Two vacancies

Component 5: Prevention and Control of Reproductive and Neonatal Diseases

Reproductive performance is the foundation of a successful food animal production system. Sows need to deliver as many healthy pigs as possible to ensure an efficient and cost effective pork production system. Cows need to deliver healthy calves to produce wholesome meat and milk. The reproductive health of poultry egg-layers and breeders directly impacts egg yield and the ability to seed broiler houses with quality stock. A multitude of infectious and metabolic diseases can decrease reproductive performance. Preventing reproductive and neonatal diseases will determine at the onset whether an animal will realize its full production potential. However, as our food animal production systems have become more efficient there has been a concomitant decrease in the reproductive performance of dairy cows and our livestock and poultry industries continue to experience significant neonatal morbidity and mortality.

The decline in fertility of dairy cows is multifactorial. Epidemiological studies indicate that factors such as dystocia, retained placenta, metritis and ovarian cysts associated with infertility are conditions that develop secondary to metabolic diseases such as hypocalcemia and ketosis. Herds suffering low pregnancy rates are generally experiencing multiple management inefficiencies including constant exposure to infectious diseases such as bovine viral diarrhea virus (BVDV) and *Neospora caninum* (Neosporosis) leading to an increase in early embryonic mortality and late term abortions. They are also common underlying metabolic diseases that further compromise immune functions and contribute to poor estrus expression and/or detection, extended anovulatory periods, and low conception rates. Hypocalcemia and negative energy balance are major metabolic challenges to the cow. Marked negative changes in energy balance and reduced immunocompetence influence gonadotropic and metabolic hormones. New research is needed to understand the metabolic effects of lactation on reproduction and understanding mechanisms linking disease to poor conception and early embryonic mortality.

The reproductive health of beef and dairy cows also impact the health of neonates. Nearly one in 11 calves born alive dies before it is weaned. Failure of passive transfer of maternal antibody and failure to develop an immune system capable of staving off common pathogens are major factors affecting morbidity and mortality of neonates. Understanding the ontogeny of the development of the immune system in neonates to a fully functional adult immune system will be critical to improve survival on farms where

exposure to infectious agents is rapidly increasing due to congregation of large numbers of animals at one site.

The ARS animal health reproductive and neonatal diseases research program will focus on three strategic areas: 1) understanding the pathogenesis of disease; 2) understanding mechanisms of disease-protection *in utero*; and 3) the development of highly effective diagnostics, vaccines, and biotherapeutics to prevent and control priority diseases. Available resources will be directed to solve problems in following key areas: eradication of bovine viral diarrhea (BVD); *Neospora caninum* (Neosporosis); and the reproductive health of the dairy cow. Additional diseases that also impact reproductive performance, neonatal health, and egg production are addressed in the following action plan research components: Porcine Reproductive and Respiratory Syndrome (**Problem Statement 4B**) and Mycoplasmosis of poultry (**Problem Statement 4C**).

Stakeholders at the September 2005 Animal Health Program Planning Workshop representing the dairy industry ranked reproductive and neonatal diseases as their second highest priority. The National Cattleman's Beef Association (NCBA) Fiscal Year 2005 Emerging Cattle Health and Issues Working Group identified BVB and Neosporosis on their list of animal disease research priorities.

Problem Statement 5A: Bovine Viral Diarrhea (BVD)

Bovine viral diarrhea virus (BVDV) causes one of the most economically devastating disease complexes of the cattle industry worldwide. The prevalence of this virus is quite high with reports of 70-90% of cattle being positive by serology. The many vaccines currently available are only marginally effective and have not provided adequate control of this costly disease. BVDV is estimated to cause $1 Billion annually in economic losses to the U.S. dairy and beef industries. These losses are reflective of respiratory and enteric diseases resulting in increased medical treatment costs and mortalities, and reproductive losses and reduced milk production. BVDV infections in cattle result in a broad spectrum of clinical diseases including embryonic death, abortion, birth defects, pneumonia and diarrhea. The BVDV causes much of its clinical damage through its immunosuppressive effects in infected animals. A critical issue impeding effective management of BVDV in cattle herds is the ability of the virus to establish a persistent infection following infection in utero. Such persistent infections are life long and the resulting persistently infected (PI) animals represent serious threats to herd mates as a source of BVDV.

The Academy of Veterinary Consultants, the American Association of Bovine Practitioners, the U.S. Animal Health Association and the National Cattlemen's Beef Association have all passed resolutions identifying the need for eradication of BVDV from the U.S. cattle herd in order to protect our stakeholder's foreign market share for cattle exports. Moreover, stakeholders at the ARS September 2005 Animal Health Workshop representing the dairy industry ranked reproductive and neonatal diseases as their second highest priority. Veterinary diagnostic laboratories across the U.S., routinely report BVDV as the primary viral pathogen isolated from cases of bovine respiratory disease.

The ARS BVDV research program will focus primarily on the reproductive aspects of the disease, in particular fetal infections and PI calves, which are the strategic control points for preventing new infections in the herd. The research will also benefit BVDV-associated respiratory and enteric diseases, which are the major thrusts of Action Plan **Components 4 and 6**, respectively.

Research Needs:
Several critical gaps exist in our scientific knowledge of BVDV disease pathogenesis. There is a need to understand what constitutes a protective immune response, particularly for fetal protection. The mechanisms of immune tolerance that lead to persistent infections *in utero* need to be defined in order to determine whether they can be prevented. The mechanisms of BVDV-induced immune suppression need to be understood as this may impede the development of effective vaccines. Improved diagnostics that enable detection of cows pregnant with persistently infected fetuses are needed. The impact of BVDV in non-bovine species on a national BVDV control and eradication program is unknown as well as interference from newly emerging BVDV variants and other pestivirus species.

Outputs:
Discovery of methods to diagnose cattle pregnant with persistently infected fetuses.
Discovery of effective diagnostics, vaccines, and biotherapeutics to support reproductive health management protocols.

Impact:
Results of this research will provide improved diagnostic tests, vaccines and scientific information to elucidate the causes of persistently infected ruminants with BVDV. Results of this research will also provide new information on how certain viruses (e.g., pestiviruses) are able to attack the host immune system, thus enabling science-based strategies for vaccine or biotherapeutic intervention. This research will provide countermeasures and integrated protocols to enable eradication or more effective prevention of BVDV in U.S. cattle herds.

Resources:
One (1) ARS CRIS project coded to National Program 103 address the research problems identified under Component 5A. Two (2) ARS scientists who are assigned to these projects include:

Ames Iowa Neil, John; Ridpath, Julia

Problem Statement 5B: Neosporosis
Neosporosis is responsible for 30-50 percent of all abortions in dairy cattle, which represents a significant loss to the dairy industry. Worldwide, it is estimated that bovine neosporosis is responsible for nearly 50% of all abortions in dairy and beef cattle herds. The disease is particularly difficult to control because once infected with the causative agent, *Neospora caninum*, cows harbor the parasite as tissue cyst stages for the remainder

of their lives. It is believed that natural immune suppression associated with pregnancy is a signal for parasite reactivation from cysts. These tachyzoite stages multiply, pass through the placenta, and infect the fetus, which may lead to abortion, stillbirth, birth of a disease calf, or birth of a clinically normal, but infected calf. In addition to placental infection, cows may become infected by oral ingestion of *N. caninum* oocysts that are shed in feces by certain canid species (i.e. dogs, coyotes). A variety of studies have shown the mechanism by which cows and dogs become infected, which stage of gestation does abortion most likely occur in infected animals, and most importantly that in an experimental disease model, sheep can be completely protected against abortion and/or transplacental infection by immunization of dams with whole *N. caninum* antigen. However, the efficacy of experimental *N. caninum* vaccines tested to date in cattle is marginal and effective disease management program are unavailable.

Research Needs:
Successful management of this disease in cattle will require a thorough understanding of neosporosis epidemiology, in particular identification of a small reservoir host (e.g. rodents), other definitive hosts (e.g. canids), and development of field pen-side diagnostic tests. Prevention of neosporosis will be achieved through the development of highly efficacious vaccines against *N. caninum* infection of adult cows, either oocyst-derived or arising from cyst reactivation. Dairy and beef herd health control programs designed to minimize exposure to *N. caninum* and integrate effective diagnostic and vaccine countermeasures will be developed.

Outputs:
Scientific information to increase our understanding of the epidemiology and transmission patterns of *N. caninum* in dairy and beef herds, and the role of dogs and wild canids and small rodents in the infection cycle.
Field diagnostic tests for rapid identification of neosporosis in dairy and beef herds.
A recombinant vaccine against *N. caninum* tachyzoites that is highly effective in preventing abortion and reactivation of tissue cysts.
Neosporosis herd health management protocols to limit exposure, prevent the transmission and spread of *N. caninum,* and prevent abortion when exposed.

Impact:
A reduction in the incidence of neosporosis-associated abortion will improve the reproductive efficiency of U.S dairy and beef herds.

Resources:
One (1) ARS CRIS projects coded to National Program 103 address the research problems identified under Component 5B. Three (3) ARS scientists who are assigned to these projects include:

Beltsville MD Dubey, Jitender; Jenkins, Mark; Tuo, Wenbin

Problem Statement 5C: Reproductive Health of the Dairy Cow

Selection for increased milk yield has significantly lowered the overall reproductive performance of dairy cattle. Pregnancy rates of lactating dairy cows per artificial insemination (AI) have decreased from 66 percent in 1951, to about 50 percent in 1975, to about 40 percent currently. Selection for high milk yield and its components have improved milk yield, but reduced daughter pregnancy rate and increased the incidence of metabolic diseases. Research shows that metabolic diseases contribute to shorter herd life, higher calving intervals, and reduced milk production in the ensuing lactation. Factors that limit fertility of lactating dairy cows include metabolic diseases, negative energy balance, toxic concentrations of urea and nitrogen, stress, and vitamin and mineral deficiencies. No less important is the impact of infectious diseases. Of particular concern are pathogens associated with late term abortions, but also frequently undiagnosed pathogens that cause insidious chronic diseases that are subclinical and impact the reproductive performance of the dairy herd. Low reproductive efficiency associated with early embryonic death or abortions due to infectious agents will only be combated when we have the tools necessary to eliminate these infections from dairy herds. Retained placentas and metritis are two disease outcomes linked to both immune dysfunction and metabolic disease that support culling decisions and reduce a dairy cow's productive lifespan. Managing a dairy farm today requires the execution of complex and expensive disease management protocols that need to be applied at critical points in the cow's production cycle: the dry, fresh, and breeding periods. Significant gaps remain in our understanding of how host genetics and the pathogenesis of infectious and metabolic diseases influence reproductive efficiency.

Stakeholders at our September 2005 Animal Health Workshop representing the dairy industry ranked reproductive and neonatal diseases as their 2nd priority.

Research Needs:
The etiology, epidemiology, and pathology of infectious and metabolic diseases that affect reproductive performance need to be identified and characterized. Genetic and phenotypic markers associated with infertility must be identified. Genetic and phenotypic markers need to be evaluated in populations segregating for reproductive health traits that may be useful in the selection of animals to increase the reproductive performance of U.S dairy herds. Factors affecting immune system function during the dry, fresh, and breeding periods need to be characterized. Effective countermeasures designed specifically to prevent and control diseases at critical control points in the dairy cow's production cycle must be developed. Cost effective reproductive health management protocols integrating effective disease control measures need to be developed.

Outputs:
Scientific information to determine the role of infectious and metabolic diseases that result in decreased reproductive performance.
Scientific information to define immune mechanisms involved in reproductive failure.
Discovery of genetic and phenotypic markers that influence fertility.
Development of diagnostics to enable marker-assisted selection programs.

Development of management tools to prevent infectious and metabolic diseases that affect reproductive efficiency.
Discovery of effective diagnostics, vaccines, and biotherapeutics to support reproductive health management protocols.

Impact:
Results of this research work will provide scientific information to elucidate the causes of reproductive diseases and provide countermeasures and integrated protocols to increase the overall reproductive performance of U.S dairy herds.

Resources:
One (1) ARS CRIS project coded to National Program 103 address the research problems identified under Component 5C. Three (3) ARS scientists who are assigned to these projects include:

Ames IA Goff, Jesse; Horst, Ronald; Reinhardt, Timothy

Component 6: Prevention and Control of 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 September 2005 Animal Health Workshop representing the dairy industry ranked Johne's disease as their 1st priority; stakeholders representing the turkey industry ranked Poult Enteric Mortality Syndrome (PEMS) as the third most important disease behind avian influenza and avian pneumovirus; and stakeholders representing the poultry broiler industry ranked enteric diseases as their 4th priority with Runting-Stunting Syndrome of broilers (RSS) the most important disease.

Problem Statement 6A: 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% 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 either asymptomatic or shedding the microorganism intermittently. In addition, host immunity to infection is mediated by Th1-type responses in early infection, with a shift to Th2-type responses in later infection. This precludes the use of a single diagnostic tool for the accurate detection of infection. In addition, 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 U.S.

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.

Outputs:
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.

Impact:
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.

Resources:
One (1) ARS CRIS project is coded to National Program 103 to address the research problems identified under Component 6A. Five (5) ARS scientists who are assigned to this project include:

Ames IA Bannantine, John; Paustian, Michael; Stabel, Judith; Two Vacancies

Problem Statement 6B: 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. Determining the cause of enteric disease in poultry has been difficult. First, definitive identification of pathogens has been challenging as many enteric viruses can not be grown in the laboratory and available virus detection tests have poor sensitivity and specificity. Secondly, enteric diseases can be caused by two or more infectious agents, and numerous agents and combinations of agents likely 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. These include 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. The ARS enteric diseases of poultry research program will use available resources to focus on enteric viruses of poultry.

Research Needs:

Characterizing the immune response during enteric infections and applying immunological and genomic approaches to the identification of host and pathogen genes involved in resistance to enteric infections.

Developing tools to study the epidemiology and genetic relationships of enteric infectious organisms.

Knowledge of the processes that regulate host response to enteric infection to develop effective strategies to prevent enteric disease.

Differential expression of genes that govern the process involved in host defense against enteric diseases. Developing tools to study the epidemiology and genetic relationships of enteric infectious agents.

Novel interventions for the prevention and treatment of enteric infections.

Diagnostic and vaccine platforms integrated in the development of cost-effective biosecurity control measures.

Outputs:

Determine and quantify factors associated with disease risk.

Determine modulators of stress in production systems that affect enteric disease development.

Discovery of cytokines and their expression profiles that govern processes involved in host defense during enteric infection.

Identify and characterize pathogens responsible for poultry enteric disease complexes.
Develop pathogen-specific markers useful for molecular or immunological detection.
Develop molecular tools to study the epidemiology and ecology of enteric pathogens.
Discover strategies that enhance the clearance of enteric pathogens.
Discover immune epitope candidates for the successful development of vaccine strategies.
Discover immunointervention strategies that prevent the development of enteric infections.

Impact:
Ability to detect pathogens responsible for enteric disease complexes.
Understand the relationship of enteric pathogens to each other and host co-evolution.
Discovery of tools that enable the prevention and control of enteric diseases.

Resources:
One (1) ARS CRIS project coded to National Program 103 addresses the research problems identified under Component 6B. Three (3) ARS scientists who are assigned to these projects include:

Athens, GA Dya, Michael J; Pantin-Jackwood, Mary; Spackman, Erica

Component 7: Prevention and Control of 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. Livestock and poultry industries are severely affected by significant losses in animal production due to lower weight gain, anemia, diarrhea, and death. Nematode infections in cattle and the cost of combating these parasites costs beef producers over $ 1 billion per year. Many parasites are invasive and exotic to the U.S and impact international trade. Most importantly, the emergence of drug resistant parasites against many commonly used pharmaceutical drugs has huge economic implications. Stakeholders at the September 2005 Animal Health Planning Workshop representing the beef industry ranked anti-parasitic drug resistance as their 5th priority; the dairy industry ranked hemoparasites as their 5th priority; the equine industry ranked diseases that impact international movement of horses such as Piroplasmosis (Equine Babesiosis) as their 1st priority; and the sheep and goat industries ranked internal parasites as their 2nd priority. The poultry broiler industry identified enteric diseases such as coccidiosis their 4th priority. The National Cattleman's Beef Association (NCBA) Fiscal Year 2005 Emerging Cattle Health and Issues Working Group identified Anaplasmosis on their list of animal disease research priorities.

Problem Statement 7A: Drug Resistant Gastrointestinal (GI) Parasitic Disease

Drug resistance has emerged as the single most important problem confronting the control of parasites in livestock worldwide. The use of drugs continue to be the primary treatment against parasites but the intensive use of these products has resulted in 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 that 20% 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 U.S is no less important. Helminthic diseases of cattle and sheep are increasing due to the ever-increasing incidence of drug resistance in parasitic nematodes. 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. The application of classical and molecular tools to rapidly and reliably differentiate these parasites will be critical to managing and controlling parasitic diseases in the face of 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, there has been documentation of increase drug resistance in nematodes of cattle. The drug resistance appears to vary according to region.

Outputs:

Develop molecular-based techniques to rapidly speciate and quantify *Eimeria* oocysts in litter samples.

Develop rapid tests to identify drug resistance markers in *Eimeria* field isolates.

Discover recombinant vaccines that are safe and effective against heterologous field challenges with mass vaccination capability to prevent outbreaks of coccidiosis in poultry farms.

Investigate and document drug resistance related to parasite species; e.g., *Haemonchus contortus*, *H. placei*, *Cooperia punctata*, *C. oncophora*, *Ostertagia ostergii*, *Nematodirus helvetianus*, and trichostrongyles.

Determine the effect of different production and management systems on the manifestation of drug resistance in sheep, dairy, cow-calf, and feedlot operations.

Identify molecular probes to better define parasite species in the field.

Identify molecular markers of drug resistance based on mode of action and measure the allele frequency of parasite genes involved in drug resistance.

Identify patterns of gene flow in nematode populations to manage drug resistance in different production systems to reduce the impact of drug resistance on productivity.

Impact:

A greater understanding of the extent and type of drug resistance in nematodes of U.S. cattle and sheep, especially as related to the type and phase of farm management.

Improved molecular probes for speciating nematodes in the farm environment, and for identifying markers of drug resistance.

Reduction in the incidence and effects of nematode infections in cattle and sheep by allowing fact-based application of appropriate anti-helminthic compounds.

Resources:
One (1) ARS CRIS project is coded to National Program 103 to address the research problems identified under Component 7A. One (1) ARS scientist who are assigned to this project include:

Beltsville MD: Hoberg, Eric

Problem Statement 7B: Hemoparasitic Diseases
Hemoparasitic diseases result in significant export and production problems for the U.S cattle and equine industries.

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 contributions of ticks to efficiency of parasite transmission. Anaplasmosis is thought to be responsible for at least 50-100,000 cattle deaths per year in the U.S with economic losses ranging from 30-60 million. Sub-clinical losses including weight gain losses, production losses, and clinical losses such as abortions, likely 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 are 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 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 U.S until the 1940’s when it was controlled by eradication of the tick vectors by intensive acaricide dipping of cattle. However, the tick vectors are present in a buffer zone along the Rio Grande, in Mexico, and in U.S territories, and pose the threat of continual reemergence into the U.S 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 U.S. 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) acaracide 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 U.S. 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. Two products from research that would alleviate this threat are safe and effective vaccines for use in the U.S (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. 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 U.S is over $1.3 billion. There is currently no babesial vaccine licensed for use in the U.S, and development of a vaccine is a high priority. *Babesia* is 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 U.S, 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.*

Equine pirosplamosis is another important tick-borne protozoal hemoparasitic disease that has tremendous impact on the movement of horses across international borders. Equine piroplasmosis is exotic to the U.S. Equine piroplasmosis results from infection by the protozoa *Babesia caballi* or *B. equi* (phylum Apicomplexa). The two organisms may infect an animal concurrently. 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, and poor exercise tolerance. *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*. The vectors for this disease in the Western Hemisphere have not been identified. *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 Anaplasmosis in areas within the U.S characterized as endemic and the discovery of parasite antigen structure associated with high transmission efficiency.

Outputs:

Determine the transmission competence of vectors within the U.S and trading partners (Canada).
Develop vaccines which prevent production losses from clinical disease and transmission (transfection technology is the center of our vaccine strategy for babesiosis).
Determine if current chemotherapeutics for *A. marginale* and *Babesia caballi* are effective in clearing persistent infections.

Impact:
Data supporting and aiding decisions on import/export restrictions and novel vaccines which prevent clinical disease and block vector borne- transmission

Resources:
Two (2) ARS CRIS projects are coded to National Program 103 to address the research problems identified under Component 7B. Six (6) ARS scientists who are assigned to these projects include:

Pullman WA	Goff, Will; Knowles, Donald; Noh, Susan; White, Stephen; Suarez, Carlos; Scoles, Glen

Component 8: Prevention and Control of Transmissible Spongiform Encephalopathies (TSEs)
Scrapie of sheep, bovine spongiform encephalopathy (BSE) of cattle, chronic wasting disease (CWD) of deer and elk, and variant Creutzfeldt-Jacob disease (vCJD) of humans are all fatal neurodegenerative disorders classified as transmissible spongiform encephalopathies (TSEs). There are no effective treatments or cure. The origin of TSEs has yet to be determined but scientific evidence indicates that the causal agents are abnormal prion proteins that induce a catalytic conversion of the normal host protein into an abnormal form. The abnormal prion proteins are transmissible and in most cases appear to be resistant to degradation. The discovery in 1996 that BSE of cattle is the cause of vCJD in people represented an unforeseen emerging zoonosis. That discovery has raised concern in the public health community that other TSEs such as CWD could evolve to cause disease in people. Although there is no evidence that CWD is zoonotic, TSEs have now been shown to be able to cross the species barrier, both experimentally and under natural conditions. The finding of two cases of BSE in the U.S in 2003 and 2004, and the continued spread of CWD, has led action and regulatory agencies to promulgate new regulations to control and eliminate the spread of animal TSEs. The 2003 BSE case was estimated to have cost the U.S. beef industry from $3.2 billion to $4.7 billion in 2004 from the loss of beef and offal exports. Stakeholders representing the wildlife, sheep, and goat industries at our September 2005 Animal Health Workshop ranked TSE research as their 1st priority; representatives of the beef industry ranked TSE research as their 6th priority. The 2005 ARS National Stakeholder (Electronic) Survey ranked BSE, CWD, and Scrapie as the 3rd, 6th, and 10th most important disease threats, respectively.

Problem Statement:

The Institute of Medicine of the National Academies has recently published a guidance document for the national prion research program. Key recommendations include funding basic research to elucidate: 1) the structural features of prions; 2) the molecular mechanisms of prion replication; 3) the mechanisms of pathogenesis of TSEs; and 4) the physiological function of the normal prion protein. In addition, the National Academies committee on TSEs recommended a comprehensive applied research program in diagnostics, testing blood for evidence of TSEs, epidemiological studies to monitor the occurrence of TSEs in human and animals, and research that will lead to strategies to prevent and treat TSEs.

Although a significant amount of research have been conducted in laboratories worldwide, we still do not understand the pathogenesis of prion diseases, nor do we understand the mechanisms that lead to TSE strains, their relevance to virulence, incubation periods, tissue distribution, genetic susceptibility, transmission, or host range specificity. Most importantly, there is currently no definitive ante-mortem or pre-clinical diagnosis tests for animals and people. ARS will integrate its large animal containment facilities and prion scientists into a national coordinated research program in pathogenesis, diagnostic discovery, and intervention. A critical step will involve establishing collaborations with scientists in the U.S and Europe that already have on-going TSE research programs to prevent duplication of efforts, maximize the efficiency of our research program, and look for opportunities to accelerate the research where feasible. A key objective will be to deliver technical information and countermeasures to enable action and regulatory agencies at APHIS, FSIS, EPA, and FDA to set science-based policies and support control programs.

Research Needs:
ARS will focus its resources around six research needs: 1) understand infectivity, tissues tropism, and pathogenesis; 2) identify determinants of host range specificity; 3) understand the molecular mechanisms of prion replication; 4) strain characterization and determinants of virulence; 5) develop ante-mortem (live) pre-clinical animal tests; and 6) discover cost effective methods of prion inactivation.

Outputs:

Develop sensitive and specific ante-mortem tests that are rapid and scaleable.

Determine the presence of TSE strains and unusual isomers of the prion protein and establish their biochemical, pathological, and epidemiological profile.

Determine the pathogenesis of TSEs, including establishing route(s) of prion migration in the host, amplification of the agent, and disease expression.

Conduct interspecies transmission studies to determine the host range specificity and resulting risk of TSEs to other animal species.

Develop enhanced rapid methods of agent detection to protect the human environment.

Develop cost effective methods of inactivating TSE agents.

Identify and characterize genotypic variations and functional genomic mechanisms associated with disease susceptibility or resistance.

Impact:

Scientific information will enable regulatory and action agencies to promulgate science-based regulatory policies.

The development of countermeasures will enhance current control and eradication programs for Scrapie and CWD and enable the prevention and containment of future occurrences of BSE worldwide.

Resources:

Three (3) ARS CRIS projects are coded to National Program 103 to address the research problems identified under Component 8. Thirteen (13) ARS scientists who are assigned to these projects include:

Albany CA	Carter, J. Mark; Hnasko, Robert; Onisko, Bruce; Silva, Christopher
Ames IA	Greenlee, Justin; Hamir, Amirali; Kehrli, Marcus; Kunkle, Robert; Nicholson, Eric; Richt, Juergen
Pullman WA	Alverson, Janet; Knowles, Donald; O'Rourke, Katherine; White, Stephen