Animal Health (NP 103) Annual Report for 2019

Introduction

The National Agriculture Statistics Service (NASS) Census of Agriculture 2017 report states the U.S. livestock industry's value of production or gross value of commodities and services produced is approximately $138 billion across all major food producing species. There are 94.4 million cattle in the United States, producing an estimated $50.2 billion and an additional $38.1 billion in milk alone. There are 73.2 million pigs in the United States that produce $19.2 billion in goods and services, while the poultry industry produces $42.7 billion and 5.4 million small ruminants produce $844 million. Animal disease outbreaks result in production losses, economic damages to producers and can have ripple effects into other parts of the economy that are dependent upon the livestock industry for consumption or production of goods.

The goal of Animal Health National Program is to protect and ensure the safety of the Nation's agriculture and food supply through improved disease detection, prevention, and control of high priority livestock diseases. These livestock diseases may already occur in the United States or may pose a threat if introduced into the country and in some cases, may also be a risk to human and public health. In order to protect animal health and public health, and safeguard the livestock industry, it is critically important that new tools such as vaccines and diagnostics are developed for the mitigation of these diseases. ARS conducts basic and applied research in the following research areas to deliver these solutions:

1. Biodefense
2. Antimicrobial Resistance
3. Zoonotic Bacterial Diseases
4. Respiratory Diseases
5. Priority Production Diseases
6. Parasitic Diseases
7. Transmissible Spongiform Encephalopathies

In Fiscal Year (FY) 2019 there were notable foreign animal disease outbreaks, and the continued spread of endemic diseases such as Chronic Wasting Disease. Newcastle disease outbreaks continued in California and African swine fever continued to be a disease of high importance globally. The costs of controlling the current Newcastle disease outbreak are not yet available; however, as of December 2019, 458 premises have been depopulated. African swine fever continued to spread through Asia leading to the culling of millions of animals and while it has not reached the United States, industry and government agencies are working to prepare for a potential outbreak. No licensed vaccine for this disease currently exists but ARS scientists have successfully transferred vaccine candidates to industry partners for further research and development and continue to share their expertise globally.

The Animal Health National program completed the 4th year of the 5-year national program cycle in 2019 and made significant accomplishments towards understanding priority diseases as well as the development of veterinary medical countermeasures to detect, prevent, control, and effectively respond to disease outbreaks.

Vision: The vision for ARS animal health research is to be a worldwide leader that delivers effective solutions to prevent and control animal diseases that impact agriculture and public health.

Mission: The mission of the Animal Health National Program (NP 103) is to conduct basic and applied research on selected diseases of economic importance to the United States livestock and poultry industries. Drs. Cyril Gerard Gay and Roxann Motroni lead the Animal Health National Program.

The Animal Health National Program currently includes 40 core research projects, with the support of 86 scientists located at nine research sites throughout the United States. The FY2019 ARS research budget for the Animal Health Program was $72.9 million with increases for Chronic Wasting Disease and the science program at the National Bio and Agro-defense Facility (NBAF). Scientists working in the program published 167 manuscripts in peer-reviewed journals and 2 book chapters. A total of nine new inventions were disclosed, six patents filed, and four new patents awarded. Additional technology transfer included 64 Material Transfer Agreements, four new Material Transfer Research Agreements, and three new Cooperative Research and Development Agreements. The NP103 program also trained 99 students and post-doctoral candidates during FY2019.

In FY2019, NBAF, which will replace the aging Plum Island Animal Disease Center (PIADC), was transferred from being owned and operated by the Department of Homeland Security to the United States Department of Agriculture (USDA). The NBAF will function within the Animal Health National Program and will provide for the first time BSL-4 capabilities to USDA and allow the program to further expand into high consequence zoonotic pathogens.

New additions to the NP 103 team in 2019 are:

Dr. Alfonso Clavijo, Director of the National Bio and Agro-Defense Facility, Manhattan, Kansas.

Dr. Fayna Diaz-Segundo, Collaborator, Plum Island Animal Disease Center, Orient Point, New York.

Dr. Cari Hearn, Research Veterinary Medical Officer, Avian Disease & Oncology Laboratory, East Lansing, Michigan.

Dr. Hao Ma, Computational Biologist, Ruminant Diseases & Immunology Research Unit, Ames, Iowa.

Dr. Emily Wynn, Post-Doctoral Fellow, joined Genetics, Breeding and Animal Health Research Unit, Clay Center, Nebraska.

The following scientists retired:

Dr. Claudio Afonso, Research Microbiologist, Exotic & Emerging Avian Viral Diseases Research Unit, Athens, Georgia.

Dr. Hong Li, Research Microbiologist, Animal Disease Research Unit, Pullman, Washington.

Dr. Eileen Thacker, Center Director, U.S. National Poultry Research Center, Athens, Georgia.

The distinguished record of these scientists is recognized world-wide and they will be missed at NP 103.

The following scientists in NP 103 received prominent awards in 2019:

Dr. Joan K. Lunney, Animal Parasitic Diseases Laboratory, Beltsville, Maryland, was inducted into the Agricultural Research Service Science Hall of Fame to honor senior agency researchers for outstanding, lifelong achievements in agricultural science and technology.

Dr. Amy L. Vincent, Virus and Prion Research, Ames, Iowa, received the Arthur S. Flemming Award for outstanding scientific achievements in the fields of animal health.

Drs. Amy L. Vincent, Crystal L. Loving, and Kelly M. Lager, National Animal Disease Center, Ames, Iowa, members of the Attenuated Swine Influenza Vaccine Team, received the USDA ARS Midwest Area Technology Transfer Award.

Research Results*:*
The following section of the report summarizes high impact research results addressing objectives in the current national program action plan components.

Component 1: Biodefense

Problem Statement 1A: Foreign Animal Diseases

African Swine Fever (ASF) Candidate Vaccines Transferred to Industry for Research and Development

Plum Island Animal Disease Center: Foreign Animal Disease Research Unit
Orient Point, New York
African swine fever (ASF) virus causes infections in domestic pigs that are often fatal and are characterized by fever, hemorrhages, ataxia and severe lethargy.

African swine fever was initially limited to sub-Saharan Africa. However, in 2007, a very virulent viral strain of ASF was introduced into the Republic of Georgia. Subsequently, the virus (Georgia 2007) started to spread to the Russian Federation, reached the European Union in 2014, and in 2018, the disease reached the world's largest pig producer, China. Thus, the disease has spread to three continents over the last decade and ASF now has an unprecedented geographical scope and poses a major threat to the U.S swine industry. There is no vaccine available for ASF and the control of the disease is strictly dependent on animal quarantine, biosecurity measures, and slaughter. This presents a major gap in the availability of veterinary medical countermeasures to effectively prevent, control, and eradicate an ASF outbreak. A notable accomplishment in 2019 was the successful development and transfer of ASF candidate vaccines by ARS scientists to five pharmaceutical companies. Important milestones included the submission of patents to the U.S Patent and Trademark Office, five U.S. Government Patent and Biological Material Licenses, and one Cooperative Research and Development Agreement (CRADA). These important milestones set the stage to begin the process of developing the first safe and effective commercial ASF vaccine.

Study Reveals Pigs Can Transmit FMD Virus Prior to Signs of Sickness

Plum Island Animal Disease Center: Foreign Animal Disease Research Unit
Orient Point, New York

Foot-and-mouth disease (FMD) virus spreads much more aggressively in pigs than previous research suggested, according to ARS scientists at the Plum Island Animal Disease Center. The new study showed that pigs were able to infect other pigs just 24 hours after being themselves infected with the FMD virus, long before showing any clinical signs. Prior to this research, it was believed that FMD transmission in pigs did not occur before visible signs of illness. Therefore, previous disease-dynamics models to predict disease impacts and estimate outbreak resource requirements did not account for the impact of preclinical transmission. Working with scientists at the Animal and Plant Health Inspection Service, Center for Epidemiology, Animal Health, ARS scientists used a mathematical modeling approach to estimate the impact of FMD preclinical transmission amongst pigs. With this new information, the models showed FMD outbreaks in the U.S. pig production sector would result in a 40-percent increase in the number of farms affected over previous estimates. That translates into 166 additional farms and more than 664,000 pigs euthanized compared to the previous models. Failure to account for information like this could make the difference between a limited, well-controlled FMD outbreak in the United States with a cost of $3 million over two months as opposed to a catastrophic nationwide epidemic with a cost of $20 billion over one year. Infectious disease modeling is a critical part of preparedness and protection of U.S. livestock. Research such as this provides critical information to help build better models to protect livestock industries from FMD.

Newcastle Disease Virus that was the Cause of the 2018 California Outbreak is Similar to the Virus that Caused the Outbreak in 2002

Southeast Poultry Research Laboratory: Exotic and Emerging Avian Viral Diseases Research
Athens, Georgia

Newcastle disease is a foreign animal disease of poultry and three outbreaks have occurred in the United States in the last century with the last outbreak currently ongoing in California. These outbreaks have cost several hundreds of millions of dollars to control. To better determine the risks presented by the current California virulent Newcastle disease virus, ARS researchers in Athens, Georgia, conducted studies to compare the virulence and transmission dynamics of the current outbreak strain with the previous virus. It was concluded that the new Newcastle disease virus presents characteristics that are consistent with a highly virulent virus that is highly similar to the California 2002 virus. This study is important to researchers and response agencies as it provides key insights into the risk of the present outbreak and demonstrates that previous studies conducted by ARS researchers on the California 2002 virus is still valid and applicable to the current outbreak.

Efficacy of Two Licensed H5 Vaccines Against Challenge with a 2015 United States H5N2 clade 2.3.4.4 Highly Pathogenic Avian Influenza Virus in Domestic Ducks

Southeast Poultry Research Laboratory: Exotic and Emerging Avian Viral Diseases Research
Athens, Georgia

Highly pathogenic avian influenza (HPAI) clade 2.3.4.4 viruses caused a major AI outbreak in poultry in the United States in 2015. Although the outbreak was controlled, vaccines were considered as an alternative control method and new vaccines were approved and purchased by the National Veterinary Stockpile for emergency use. ARS researchers in Athens, Georgia, evaluated the efficacy of two of these vaccines in protecting Pekin ducks against challenge with an H5N2 HPAI poultry isolate. A recombinant alphavirus-based vaccine and an inactivated adjuvanted reverse genetics vaccine (originally developed by ARS and transferred to a commercial partner for manufacturing and stockpiling) were used to immunize the ducks. Both vaccines, regardless of the vaccination strategy used, reduced or prevented disease and virus shedding after challenge. This information is important to prepare and control avian influenza in domestic ducks should another outbreak with H5N2 clade 2.3.4.4 occur in the United States.

ARS Workforce Development: Creating the Next Generation of Biodefense Researchers

National Bio and Agro-Defense Facility
Manhattan, Kansas

In FY19, ARS created Workforce Development training agreements with the Auburn University, the University of Connecticut and the University of Minnesota. All three Universities are new NBAF training partners with ARS. These agreements will support 4 new (FY19) trainees in immunology/vaccinology, epidemiology and disease pathogenesis. There are 7 trainees currently at Mississippi State University and Kansas State University (agreements established in FY17 and FY18). ARS scientists are collaboratively involved in these research training projects. ARS held a research symposium for the workforce development trainees, and their USDA and University mentors in Manhattan, Kansas, on August 27, 2019.

Problem Statement 1B: Emerging Diseases

Publication of a Unified Classification and Naming System for New Emerging Newcastle Disease Viruses

Southeast Poultry Research Laboratory: Exotic and Emerging Avian Viral Diseases Research
Athens, Georgia
Newcastle disease virus has high sequence variability that often is unique by country or region of the world. Several classification systems have previously been published that have established unique genotypes to describe these differences, but because of a lack of strict rules governing the establishment of new genotypes, a single system has not been widely adopted. ARS researchers in Athens, Georgia, led an effort to establish a consortium of 23 reference and research laboratories to develop new and consistent naming rules using the complete fusion gene sequence to establish a consistent naming system, and the consortium published these results. This new nomenclature system is likely to become the de facto standard for genotype naming for Newcastle disease viruses.

Epizootic Hemorrhagic Disease Virus (EHDV) Infection Affects Sensory and Neural Tissues in Culicoides Midges

Center for Grain and Animal Health Research: Arthropod Borne Animal Disease Research Unit
Manhattan, Kansas
Epizootic Hemorrhagic disease is an emerging disease caused by a virus transmitted by culicoides nsects, commonly referred to as midges, that has caused significant economic losses in the captive deer industry. This virus has also infected cattle during disease outbreaks resulting in additional economic losses to the cattle industry. The midges get infected by feeding on an animal with the virus, and then transmit the virus through subsequent bites. With collaborators at Clemson University and University of South Carolina, ARS scientists identified key changes in female midge gene expression profiles occurring during early infection with EHDV. Genes that had decreased expression included those for sensory functions (especially vision), behavior, learning, and memory. Genes that had increased expression included those for immune processes, odor, and light detection. These results suggest that EHDV infection may have a significant effect on sensory and neural tissues of midges and suggest a change needed in the use of lamps that use light wavelength to optimally attract and trap infected insects during outbreaks, which is important to researchers as well as industries interested in trapping the insects as a means of disease control.

A New United States Swine Pathogen Database

National Animal Disease Center: Virus and Prion Research
Ames, Iowa
In recent years, several deadly viral diseases of pigs have emerged in the United States causing hundreds of millions of dollars in economic damages. In order to effectively respond to these diseases or detect new disease incursions or viral variants, it is critical to have a database of currently circulating viral genetic sequences and associated tools to

analyze the sequences. ARS scientists in Ames, Iowa, created such a database (https://swinepathogendb.org) for porcine reproductive and respiratory syndrome virus (PRRSV), Senecavirus A (SVA), and porcine epidemic diarrhea virus (PEDV) using nucleotide sequences and related metadata found in GenBank, part of the United States National Center for Biotechnology Information, and from clinical cases detected by key veterinary laboratories. Presently, the South Dakota Animal Disease Research and Diagnostic Laboratory, the Iowa State Veterinary Diagnostic Laboratory and the Kansas State University Veterinary Diagnostic Laboratory have submitted over 2000 sequences. A suite of web-based tools allows stakeholders, researchers and veterinarians to quickly search for genetic sequence information, identify similar viruses, and browse virus genomes to inform research and control efforts. Databases such as these will greatly increase researchers' understanding of endemic circulating viruses as well as speed response efforts by quickly identifying new viral variants.

Component 2: Antimicrobial Resistance

Problem Statement 2A: Ecology of Antimicrobial Resistance

Antimicrobial Resistance Distribution Differs Among Methicillin Resistant Staphylococcus aureus (MRSA) Isolates from Health Care and Agricultural Sources

National Animal Disease Center: Virus and Prion Research
Ames, Iowa

Antimicrobial resistance is an expanding public health concern. Since the discovery of livestock associated MRSA (LA-MRSA), public health concerns have increased surrounding the potential of LA-MRSA isolates to serve as a reservoir for antimicrobial resistance genes. ARS researchers at Ames, Iowa, compared swine associated LA-MRSA ST5 and human clinical MRSA ST5 isolates for antimicrobial susceptibilities and genes associated with antimicrobial resistance. Swine associated LA-MRSA ST5 isolates showed resistance to fewer antibiotics than clinical MRSA ST5 isolates from humans with no swine contact. Distinct genomic antimicrobial resistance elements were harbored by each subgroup, with little overlap in shared antimicrobial resistance genes between swine associated LA-MRSA ST5 and clinical MRSA ST5 isolates. These results are important to public health officials and policy-makers since the results demonstrate swine associated LA-MRSA ST5 and human clinical MRSA ST5 isolates are separate and distinct suggesting that swine do not play a major role in maintaining a MRSA ST5 reservoir affecting humans.

Antibiotics Alter the Normal Swine Respiratory Microbial Community

National Animal Disease Center: Virus and Prion Research
Ames, Iowa

Antibiotic stewardship is of the utmost importance to improve animal health outcomes and prevent selection of antimicrobial resistance. There is increasing evidence of the important role the normal respiratory microbial community (microbiota) plays in shaping immune and respiratory health. However, there is little knowledge on the effects of antibiotics on the swine respiratory microbiota. Oxytetracycline is a broad-spectrum antibiotic that is used for treatment of bacterial respiratory disease in swine. ARS researchers at Ames, Iowa, showed the respiratory microbiota diversity decreased in response to oxytetracycline administration. In addition, giving the antibiotic in the feed had a greater and longer lasting impact on the nasal microbiota than the injectable route. There were also increased abundances of some pathogenic bacteria and decreased abundances of normal respiratory resident bacteria after antibiotic treatment. These results highlight the need to further assess how these changes can ultimately affect the animal's respiratory health and risk to disease.

Subinhibitory Concentrations of Antibiotics Commonly used to Treat Swine Increase Streptococcus suis (S. suis) Biofilm Formation.

National Animal Disease Center: Virus and Prion Research
Ames, Iowa

S. suis is the leading bacterial swine pathogen worldwide causing a wide variety of clinical presentations in pigs ranging from asymptomatic carriage to lethal systemic disease. *S. suis* is also a zoonotic pathogen capable of causing invasive disease in humans. A barrier towards the development of improved vaccines or interventions for *S. suis* infections is a gap in understanding the factors contributing to persistence in the host, in which colonized pigs continue to shed and transmit *S. suis*. Biofilms are adherent communities of bacteria that are protected from common mechanisms that the body uses to clear bacteria and are considered a key factor contributing to chronic or persistent bacterial infections. Routine management practices involve treating all pigs in the same pen or barn with appropriate antibiotics upon observing any pig exhibiting clinical signs associated with a bacterial infection. ARS researchers at Ames, Iowa, studied the effects of sub-inhibitory concentrations of antibiotics commonly used by the swine industry on the development of *S. suis* biofilms and found amoxicillin, lincomycin, and oxytetracycline increase biofilm formation while bacitracin, carbadox, chlortetracycline, enrofloxacin, gentamicin, neomycin, sulfadimethoxine, tiamulin, and tylosin did not. Collectively, data demonstrate that exposure to some commonly used antibiotics contributes to increased biofilm formation of *S. suis*, thereby potentially increasing survival and persistence within the respiratory tract of swine and increasing transmission dynamics among animals. This data is critical for proper selection of antibiotics for successful treatment of swine bacterial diseases while minimizing potential collateral consequences.

Problem Statement 2B: Alternatives to Antibiotics

Enhanced Understanding of Mechanisms of Action of Antibiotic Growth Promoters

Beltsville Agricultural Research Center: Animal Biosciences and Biotechnology Laboratory
Beltsville, Maryland

Although antibiotic growth promoters (AGPs) have been widely used globally to make a significant contribution to the expansion of modern animal agriculture, underlying modes of action of AGPs still remain unknown. In 2017, the Food and Drug Administration released the Veterinary Feed Directive that eliminated the use of antibiotics for growth performance- making it more imperative to understand how AGPs work, so rationally-designed antibiotic alternatives can be developed and produced to improve growth. ARS scientists used "omics" technology to identify biochemical pathways that are associated with the effects of AGPs. The changes in the levels of intestinal small molecular weight metabolites provided distinctive biochemical signatures unique to each antibiotic-supplemented group. These biochemical signatures were characterized by increases in the levels of metabolites of amino acids, fatty acids, nucleosides and vitamins. These results enhance our understanding of the mode of AGP action and provide the framework for future studies to identify natural chemical compounds as antibiotic alternatives to improve poultry growth performance without the use of in-feed antibiotics.

Component 3: Zoonotic Bacterial Diseases

Problem Statement 3A: Brucellosis

Understanding and Detecting Brucella abortus Vaccine RB51 Shed in Milk

National Animal Disease Center: Infectious Bacterial Diseases Research
Ames, Iowa
Brucella abortus is a highly contagious bacterial pathogen for livestock and humans and is classified as a select agent because of its potential use as a bioweapon. In the United States, a vaccine strain called RB51 is routinely used to vaccinate female calves to prevent infection and limit human exposures. Over the past two years, people in at least 3 states have been infected with the *Brucella abortus* RB51 vaccine strain after drinking unpasteurized milk. ARS scientists in Ames, Iowa, collaborated with the Centers for Disease Control and Prevention and the Animal and Plant Health Inspection Service to better understand why some cattle vaccinated as calves shed RB51 in their milk as adults. Their results showed cattle shedding RB51 in milk have a different type of immune response than those animals that do not shed RB51 in their milk. Further, ARS developed an ELISA assay which can detect cattle shedding RB51 in milk. Due to the widespread use of RB51 vaccination in cattle, this work is critical to protect public health since it provided a diagnostic assay for detecting cattle shedding RB51 in milk and provided insights into why some cattle become persistently infected with it.

Problem Statement 3B: Leptospirosis

Characterization of Treponeme Associated Hoof Disease in Free-ranging Elk

National Animal Disease Center: Infectious Bacterial Diseases Research
Ames, Iowa
ARS scientists from Ames, Iowa, collaborated with State officials and university scientists in characterization of hoof disease in free-ranging elk (*Cervus elaphus*) in southwestern Washington State, which continues to spread throughout the region with some herds showing 20 to 90 percent of animals being affected. In adult elk, lesions of hoof overgrowth, sole ulcers, and sloughed hoof capsules are observed in addition to higher mortality. A field study was conducted to characterize the pathogenesis of this disease. Lesions were most severe in elk at 9 months of age and mimicked lesions in adult elk, whereas elk examined at 3 months of age had the mildest lesions and evidence of treponemes, which are spirochete bacteria that are in the same family as leptospira. Data suggest that treponeme-associated hoof disease (TAHD) in free-ranging elk is a debilitating and progressive condition that is similar to digital dermatitis in cattle and

sheep. This research provides the critical insight that treponemes cause hoof disease in elk and also informs research on the pathogenesis of digital dermatitis in cattle and sheep, which may lead to intervention strategies. The research with elk is also of importance to the wildlife community and State animal health officials.

Component 4: Respiratory Diseases

Problem Statement 4A: Bovine

Systems biology Tool for the Analysis of Agriculturally Important Bacteria

U.S. Meat Animal Research Center: Genetics, Breeding, and Animal Health Research
Clay Center, Nebraska

One modeling approach to systems biology is the computational modeling of genes, their interactions, and the influence of the environment on the system. Until now, microbiologists and other researchers working on bacteria-related problems in animal disease, food safety, bioengineering and other agricultural domains were not able to efficiently create holistic systems biology models. Through a SCINet collaborative research initiative including ARS researchers in Clay Center, Nebraska, Iowa State University, and industry collaborators; the Pathway Tools systems biology analytical platform was customized to run on Amazon Web Services (AWS) to host systems biology models of bacterial field isolates sequenced and assembled by the USDA. The primary beneficiaries of this resource are researchers and others wishing to use, create, and publish systems biology models that relate bacterial genes to key bacterial functions or physical traits. These models will assist in generating evidence-based strategies to mitigate the effects of bacterial infection, improve food safety protocols, and promote rational, focused solutions to bacteria-related problems in agriculture.

Leukotoxin Susceptibility in Cattle

U.S. Meat Animal Research Center: Genetics, Breeding, and Animal Health Research
Clay Center, Nebraska

Bovine respiratory disease is the most significant illness in U.S. cattle and a major source of economic loss, exceeding a billion dollars annually. When bacteria invade the lungs, they secrete a toxin that binds to a receptor on bovine white blood cells and kills them, causing rapid inflammation, bleeding, and fluid accumulation in the air sacs of the lungs. This causes a severe and sometimes fatal pneumonia. ARS researchers at Clay Center, Nebraska, used a genome sequencing approach to identify differences in the naturally occurring toxin receptor in more than 1,000 cattle from 46 breeds. One of these receptor variants was 30-times more sensitive to the toxin. These results suggest that certain cattle may be at increased risk for toxin-related respiratory disease. This also provides new possibilities for disease intervention including: identifying high-risk animals through genetic testing, selectively breeding for less susceptible animals, and developing non-antibiotic treatments that may neutralize the bacterial toxin.

Problem Statement 4B: Porcine

***A New United States Swine Pathogen Database* (https://swinepathogendb.org).**

National Animal Disease Center: Virus and Prion Disease Research
Ames, Iowa

In recent years, several deadly viral diseases of pigs have emerged in the United States causing hundreds of millions of dollars in economic damages. In order to effectively respond to these diseases or detect new disease incursions or viral variants, it is critical to have a database of currently circulating viral genetic sequences and associated tools to analyze the sequences quickly and readily available to the research and diagnostic community. ARS scientists in Ames, Iowa, created such a database for porcine reproductive and respiratory syndrome virus (PRRSV), Senecavirus A (SVA), and porcine epidemic diarrhea virus (PEDV) using nucleotide sequences and related metadata found in GenBank, part of the United States National Center for Biotechnology Information, and from clinical cases detected by key veterinary laboratories. Presently, the South Dakota Animal Disease Research and Diagnostic Laboratory, the Iowa State Veterinary Diagnostic Laboratory and the Kansas State University Veterinary Diagnostic Laboratory have submitted over 2000 sequences. A suite of web-based tools allows stakeholders, researchers and veterinarians to quickly search for genetic sequence information, identify similar viruses, and browse virus genomes to inform research and control efforts. Databases such as these will greatly increase researchers' understanding of endemic circulating viruses as well as speed response efforts by quickly identifying new viral variants.

Component 5: Priority Production Diseases

Problem Statement 5A: Johne's Disease

New Diagnostics for Johne's Disease

National Animal Disease Center: Infectious Bacterial Diseases Research
Ames, Iowa

Improved diagnostic tests are needed to aid producers in controlling this disease. Using a protein array, scientists in Ames, Iowa, identified patterns of serological reactivity in cows with Johne's disease. Some antigens induced antibody responses throughout disease stages, where responses to other antigens were detected late in the course of disease, or early in infection with a decline in responses at later stages of disease. The most promising proteins identified from the protein array were tested by ELISA and a multiplex bead assay and distinguished infected from non-infected healthy animals with sensitivities ranging from 65 percent to 93 percent. Some of the proteins had better sensitivity on clinical samples when compared to a commercially available Johne's disease diagnostic tests. These data will be of interest to livestock producers, veterinarians, and diagnostic laboratories who want more accurate tests identifying animals infected with Johne's disease.

Problem Statement 5D: Bovine Mastitis

The Effect of 50 Years of Breeding on the Ability of Holsteins to Fight Mastitis

National Animal Disease Center: Ruminant Diseases and Immunology Research
Ames, Iowa

Dairy cow breeding and management has been able to dramatically increase milk production and at the same time reduce the number of cows in production. However, since the early 1970s, some researchers have expressed concern that the selective breeding for milk production could result in an associated increase in health problems. The University of Minnesota has maintained a herd of dairy cows with sires that reliably breed for the average milk yield seen in 1964. In collaboration with researchers at the University of Minnesota, ARS researchers at Ames, Iowa, have shown that Holsteins with genetics from 1964 were able to clear an experimental mastitis challenge with *Escherichia coli* better than modern Holsteins. This work shows that selective breeding for milk production is correlated with poorer immune defense against *E. coli* mastitis.

Component 6: Parasitic Diseases

Problem Statement 6A: Gastrointestinal (GI) Parasitic Diseases

Comparative Genomics of Major Parasitic Worms

Beltsville Agricultural Research Center: Animal Parasitic Disease Laboratory
Beltsville, Maryland

Parasitic nematodes (roundworms) and platyhelminths (flatworms) cause debilitating chronic infections of humans and animals, decimate crop production and are a major impediment to socioeconomic development. ARS scientists in Beltsville, Maryland, and University collaborators undertook a major survey of genes that modulate host immune responses, enable parasite migration though host tissues or allow parasites to feed. They identified extensive lineage-specific differences in core metabolism and protein families that have historically been targeted for drug development. From wide-ranging analyses involving computer modeling and simulations, they identified and prioritized new potential drug targets and compounds for testing. This study is the broadest and most comprehensive comparative study to date utilizing the genomes of parasitic and non-parasitic worms, providing a transformative new resource for the research community to understand and combat the diseases that parasitic worms cause.

Diverse Mechanisms Contribute to Parasite Resistance in Sheep

Beltsville Agricultural Research Center: Animal Parasitic Disease Laboratory
Beltsville, Maryland

In collaboration with Commonwealth Scientific Industrial Research Organization (CSIRO) scientists in Australia, ARS scientists in Beltsville, Maryland, compared resistant and susceptible sheep lines in their responses to parasite infection. These source populations are the result of efforts from over 40 years of selective breeding. The expression of nearly one thousand genes differed among resistant and susceptible animals in response to a primary infection. Different mechanisms, including coagulation, complement cascades, and tissue repair, contribute most to resistance in distinct lines of sheep. These findings elucidate fundamental aspects of livestock immunology and will support the development of genetically resistant sheep via selective breeding.

International Agreement on Diagnosing Trichinella

Beltsville Agricultural Research Center: Animal Parasitic Disease Laboratory
Beltsville, Maryland

Trichinellosis was once a major concern for pork safety, and remains a concern for consumers of wild game, which might be infected with one or more of several parasite species. Discerning which of these species is present aids epidemiological studies and outbreak control, but they all look alike, creating a need for genetic diagnostic methods. A simple and effective diagnostic assay developed by ARS researchers in Beltsville, Maryland, was endorsed by the International Commission on Trichinellosis (ICT) for use in all outbreaks and whenever Trichinella has been found in consumable foods. This will assist food inspectors and veterinarians to better understand and manage an important zoonotic disease.

Problem Statement 6B: Hemoparasitic Diseases

Transgenic Babesia bovis Lacking 6-Cys Sexual-stage Genes as the Foundation for a Non-transmissible Live Vaccine Against Bovine Babesiosis

Animal Disease Research Unit
Pullman, Washington

Babesia bovis, a tick-borne parasite responsible for bovine babesiosis has a complex life cycle including sexual development in its *Rhipicephalus microplus* tick vector. Understanding the molecular mechanisms involved in sexual development in ticks may provide a new approach for developing future-generation transmission blocking vaccines. Recently discovered sexual markers 6-CysA and 6-CysB genes of *B. bovis* were found to be expressed during the tick-stage of *B. bovis*. ARS scientists hypothesized that disruption of both 6-CysA and 6-CysB in *B. bovis* would result in unaltered ability of the parasite to invade and grow in red blood cells but lose the ability to transmit to ticks. Parasites deficient in genes required for sexual reproduction could be the foundation for genetically-defined, non-transmissible live vaccines against bovine babesiosis that could help reduce the burden of disease globally.

Component 7: Transmissible Spongiform Encephalopathies

Problem Statement 7A: Pathobiology of Prion Strains

Milk from Goats with Scrapie is Highly Infectious

Animal Disease Research Unit
Pullman, Washington

The continued presence of scrapie in the United States costs sheep producers an estimated $20 million annually, especially in unrealized exports. Since animals harbor infection for several years before developing clinical signs, it is imperative for eradication efforts to identify and mitigate transmission risks. ARS researchers at

Pullman, Washington, have determined that milk from infected goats can be highly infectious to newborn lambs and goat kids. Critically important was that transmission occurred after newborns consumed only about one gallon of milk. This knowledge informs producers that even limited exposure of newborn lambs and goat kids to scrapie-infected goat's milk can result in their flock acquiring scrapie and helps disease investigators better assess sources of flock exposure.

Problem Statement 7C: Diagnostics, Detection, and Prevention

A Method for Long Term Storage of Prion Protein

National Animal Disease Center: Virus and Prion Research
Ames, Iowa
Prion diseases include Scrapie, Chronic Wasting Disease and mad cow disease. Recent advances in prion disease diagnostic methods include the amplification of abnormal prion proteins in a sample to enable the early detection of the disease. One such technique, known as RT-QuIC, requires a steady supply of freshly purified prion protein, which necessitates constant production that is not sustainable in a diagnostic laboratory setting. ARS researchers developed a method to dry and preserve the prion protein for long term storage (months or years). This allows for production of the prion protein in larger quantities, and it can be shipped to diagnostic laboratories facilitating widespread use of RT-QuIC as a diagnostic method.

Immune Reagents

One of the major obstacles to advancing animal health research is the lack of reagents available to study the immune system of the major livestock species, as reagents are more commonly available for humans and mice and may not be cross-reactive. In 2010, USDA-NIFA started the Veterinary Immune Reagent Network (https://www.umass.edu/vetimm/) with the goal of producing these reagents for the greater scientific community.

USDA ARS scientists in Beltsville, Maryland, led the effort for the development of swine and poultry reagents for the network and their accomplishments are highlighted below.

New Tools for Swine Immunology

Sophisticated immune tools are needed to analyze disease and vaccine responses, yet those are limited for pigs. ARS scientists in Beltsville, Maryland, worked with a commercial partner to clone and express immune proteins using yeast. A second commercial partner developed monoclonal antibodies (mAbs) to these proteins which were then characterized in collaboration with university partners at Ohio State and Tennessee State Universities. Panels of mAbs reactive with 7 different swine immune proteins were characterized and transferred to commercial partners. These antibodies provide new assays to quantitate each protein in body fluids as well as to measure their intracellular expression. Tools and reagents generated by this project are now available and being used, the world-over, for studying swine immunity and disease.

Development of Poultry Immunology Tools

The lack of poultry immune reagents and immunoassays for the detection and evaluation of cell-mediated immunity in chickens hinders progress in developing antibiotic alternative strategies including vaccines. To address these technical gaps, scientists in Beltsville, Maryland, have identified and developed new immune reagents that will be critical for measuring host immune response to infectious agents and to evaluate novel antibiotic alternatives to replace antibiotics in poultry. During this reporting period, we have completed 9 target chicken proteins in monoclonal antibody (mAb) development which are critical in measuring host innate immunity in poultry. These new murine hybridoma cell lines which secrete mAbs that specifically detect chicken cytokines and chemokines including IL4, IL10, IL13, CCL4, CCL5, IL7 and granzyme A have been characterized and more importantly, detection assays for many chicken immune proteins in sera have been developed. Availability of these new immune tools will significantly advance avian immunology, facilitate the development of vaccines and support the fundamental study of host immune response against various infectious diseases.