Animal Health (NP 103) Annual Report for 2020

Introduction

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.

The goal of the Animal Health National Program is to protect and ensure the safety of the Nation's agricultural animals and economy, the food supply and public health through improved disease detection, prevention, and control of high priority livestock diseases. Animal production makes a big contribution to the agricultural economy. 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.

From a One Health perspective, protecting animal and public health means protecting against diseases, some of which are zoonotic. These livestock diseases may be domestic and already occur in the United States or may be foreign animal diseases that pose a threat if introduced into the country; in either cases, zoonotic diseases are a risk to human and public health. In order to protect animal 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) 2020 there were notable foreign animal disease outbreaks, and the continued spread of endemic diseases such as Chronic Wasting Disease. 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 new vaccine candidates to industry partners for further research and development and continue to share their expertise globally.

The Animal Health National program is completing the 4th year of the 5-year national program cycle in 2020 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.

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 FY2020 ARS research budget for the Animal Health Program was $81.6 million with increases for African swine fever, chronic wasting disease, cattle fever tick, bovine pleuropneumonia, and the science program at the National Bio and Agro-defense Facility. Scientists working in the program published 180 manuscripts in peer-reviewed journals and 16 book chapters. A total of 11 new inventions were disclosed, nine patents filed, and six new patents awarded. Additional technology transfer included 51 Material Transfer Agreements and 12 new Material Transfer Research Agreements. The NP103 program also trained 91 students and post-doctoral candidates during FY2020.

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 2020 are:

Dr. Miranda Bertram, Clinical Studies Veterinary Medical Officer, joined the Foreign Animal Disease Research Unit, Orient Point, New York

Dr. Eric Cassmann, Research Veterinary Medical Officer, joined the Virus and Prion Research Unit, Ames, Iowa

Dr. Samantha Hau, Scientist, joined the Virus and Prion Research Unit, Ames, Iowa

Dr. Carly Kanipe, Veterinary Medical Officer, joined the Infectious Bacterial Diseases of Livestock Research Unit, Ames, Iowa

Dr. Bethany McGregor, Research Entomologist, joined the Arthropod-Borne Animal Diseases Research Unit, Manhattan, Kansas

The following scientists in NP 103 received prominent awards in 2020:

Dr. John Dunn, Endemic Poultry Viral Diseases Research Unit, Athens, Georgia, received the American Association of Avian Pathologists P.P. Levine Award as the senior author of the best paper published in the journal *Avian Diseases* for the year 2019.

Dr. Shollie Falkenberg, Ruminant Diseases and Immunology Research Unit, Ames, Iowa, received the USDA Agricultural Research Service Midwest Area Early Career Research Scientist of the Year Award.

Dr. Kelly Lager, Virus and Prion Research Unit, Ames, Iowa, received the USDA Agricultural Research Service Distinguished Senior Research Scientist of the Year Award for outstanding research in swine disease pathogenesis research leading to development of improved vaccines and diagnostic tests used for control of swine diseases.

Dr. Hyun Lillehoj, Animal Biosciences & Biotechnology Laboratory, Beltsville, Maryland, was recognized for her career achievements by the USDA Agricultural Research Service as part of Women's History Month, March 2020.

Dr. David Swayne, Exotic & Emerging Avian Viral Diseases Research Unit, Athens, Georgia, was inducted into the USDA Agricultural Research Service Hall of Fame for outstanding research on pathobiology, vaccines and vaccination, and food safety of avian influenza that have brought ARS international impact and recognition.

Dr. Amy Vincent, Virus and Prion Research Unit, Ames, Iowa, was 1 of 2 veterinarians elected to the National Academy of Medicine for groundbreaking research that led to improved vaccines and surveillance for swine influenza, characterization of vaccine associated enhanced disease in a swine influenza model, and characterization of pandemic potential for swine influenza viruses.

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

Development of a Safe and Effective African Swine Fever Virus Vaccine -ASFV-G-deltaI177L

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

African Swine Fever (ASF) is a devastating and highly lethal disease of pigs for which there are no commercial vaccines. One of the most significant knowledge gaps that has hindered scientists from developing a safe and effective ASF vaccine is the lack of genomics information on the function of the virus's 150 genes. ARS scientists at the Plum Island Animal Disease Center have successfully developed genetic engineering techniques that systematically delete genes from the ASF viral genome to determine their function. Pathogenicity studies in pigs with these altered viruses has led to the discovery of essential genes for ASF viral replication, host immune evasion, and determinants of virulence. The identification of these genes provides potential targets to enable the rationale design of gene-deleted vaccines that are safe and efficacious. The most recent vaccine candidate is the discovery of a genetically engineered gene-deleted live attenuated vaccine strain called ASFV-G-delta I177L. This vaccine strain was shown to be safe and efficacious, exceeding what has previously been achieved with other ASV vaccine candidates. For the first time, the ASFV-G-delta I177L vaccine was shown to fully protect pigs against ASF with a low dose of vaccine virus. The safety characteristics of the vaccine include no adverse events even when high doses of the vaccine were administered to pigs. A patent covering the development ASFV-G-delta I177L was filed and several commercial partners have initiated the process of licensing ASFV-G-delta I177L. Importantly, ARS scientists have established a research agreement with one of these companies to initiate the development of the vaccine.

Discovery of an Established Cell Line to detect African Swine Fever Virus

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

African Swine Fever Virus (ASFV) field isolates only replicate in primary cultures of swine macrophages, which makes it difficult and time consuming to screen field isolates for virus and requires a herd of healthy donor pigs to maintain a supply of primary macrophages. The need for a herd of healthy donor pigs make swine macrophage cultures inaccessible for most of the diagnostic laboratories trying to diagnose infectious ASFV in suspect field samples. ARS scientists at the Plum Island Animal Disease Center in Orient Point, New York, discovered a cell line of monkey origin, Ma-104, that was highly

susceptible to infection with field isolates of ASFV. Ma-104 cells can be readily infected by all ASFV isolates tested and the sensitivity of detection was just below of that of the gold standard, primary swine macrophage cultures, and above the sensitivity of conventional nucleic acid detection methods. This discovery is of paramount importance for ASFV diagnostics as it will enable diagnostic laboratories worldwide to perform detection of ASFV using a readily available and easy to grow cell line.

Duration of Contagion of Foot-And-Mouth Disease Virus in Infected Live Pigs and Carcasses

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

Data-driven modeling of incursions of high-consequence foreign animal diseases is a critical component of veterinary preparedness. However, simplifying assumptions and excessive use of estimates to compensate for gaps in available data may compromise the accuracy of model outcomes. Accordingly, ARS scientists at the Plum Island Animal Disease Center conducted a study to address two major gaps in current knowledge of foot-and-mouth disease virus (FMDV) pathogenesis in pigs: 1) the end (duration) of the infectious period and 2) the viability of FMDV in decaying carcasses. By serial exposure of sentinel groups of pigs to the same group of donor pigs infected by FMDV, it was demonstrated that infected pigs transmitted disease at 10 days post infection (dpi), but not at 15 dpi. Assuming a latent period of 1 day, this would result in a conservative estimate of an infectious duration of 9 days, which is considerably longer than suggested by a previous report from an experiment performed in cattle. Airborne contagion was diminished within two days of removal of infected pigs from isolation rooms. FMDV in muscle was inactivated within 7 days in carcasses stored at 4ºC. By contrast, FMDV infectivity in vesicles from intact carcasses stored under similar conditions remained remarkably high until the study termination at 11 weeks post-mortem. This information may be used to update models used for foot-and-mouth disease outbreak simulations involving areas of substantial pig production.

Swine Influenza A Viruses and the Tangled Relationship with Humans

Virus and Prion Diseases Research Unit, National Animal Disease Center
Ames, Iowa

All circulating swine Influenza A virus (IAV) have gene segments derived from human seasonal IAV, and these swine IAV have the potential to be introduced back into the human population if they are substantially different from current human seasonal strains. ARS scientists at the National Animal Disease Center in Ames, Iowa, quantified the global genetic diversity of swine IAV circulating from 2016 to present, and the genetic diversity of swine IAV in the United States over the past 6 months. The circulating swine IAV diversity was compared to human IAV vaccines and current candidate vaccine viruses (CVV) that are used in pandemic preparedness. Representative strains were characterized to determine whether human vaccine strains or CVV strains would provide protection against these swine IAV. Only 7 of the 29 distinct circulating genetic virus clades were covered by CVV strains; and the degree to which those vaccines provide

protection is uncertain given observed genetic differences. This work demonstrated that controlling IAV in swine populations is a critical process in pandemic preparedness, and objectively identifying vaccine strain candidates for the benefit of public health pandemic preparedness is important.

Contamination of Eggs with Avian Influenza Virus Laid by Infected Hens
Exotic and Emerging Poultry Viruses Research Unit, U.S. National Poultry Research Center, Athens, Georgia

Effective recovery from an avian influenza (AI) outbreak requires reliable knowledge of the risk for transmitting the virus on or in eggs so the risk can be mitigated. However, data on AI virus contamination from eggs is almost exclusively from field reports where the timeframe of infection is not clearly known. To understand the extent that eggs laid by infected hens could be contaminated with AI virus, ARS scientists at the U.S. National Poultry Research Center in Athens, Georgia, infected hens in production with either low pathogenicity (LP) or highly pathogenic (HP) AI viruses and their eggs were collected and tested for the presence of virus on the eggshell or in the egg contents. The study showed that eggs could be contaminated with either LP or HP AI virus, but the levels of virus and numbers of infected eggs were much higher with the highly pathogenic avian influenza (HPAI) virus. These data show that eggs need to be considered as a source of virus that could potentially spread AI unless sanitization measures are employed.

Problem Statement 1B: Emerging Diseases

Improved Computer Modeling to Predict Susceptibility of Different Species to Infection with Severe Acute Respiratory Coronavirus 2 (SARS-CoV2)
Virus and Prion Diseases Research Unit, National Animal Disease Center
Ames, Iowa

As a group of obligate pathogens, viruses need to enter a cell to replicate. Viral entry is a process that begins with attachment between a virus protein and a cell receptor(s), which allows for the virus to be internalized into the cell. Once inside the cell, the virus initiates replication starting the race between host immunity and a productive infection. For coronaviruses, the spike protein protruding from the viral surface is responsible for cell receptor binding and mediating viral entry. Several groups have reported that SARS-CoV2 uses the same angiotensin-converting enzyme 2 (ACE2) as the primary receptor for cell attachment, similar to SARS-CoV discovered in 2003. SARS-CoV-2 has been shown to have higher receptor affinity to human ACE2, which may contribute to the apparent faster spread of human SARS-CoV2 infection. The susceptibility of both wild and domestic animals to SARS-VoV-2 and the potential for zoonotic transmission is a public health concern that involves two aspects: 1) screening to identify the animal species that serve as a virus reservoir originally passing SARS-CoV2 to humans; and 2) the existing risk of infected people to pass the virus to animals, particularly the domestic species, thus potentially forming into an amplifying zoonotic cycle to worsen SARS-CoV2 evolution and prevalence. ARS researchers at the National Animal Disease Center in Ames, Iowa, and collaborators at Tennessee State University evaluated cross-species

ACE2 genetic diversity in the regulation of ACE2 expression and functionality to determine the cell tropism and susceptibility of different animal species to SARS-CoV2. The analysis was used to predict the potential of livestock transmission of SARS-CoV2, and it also revealed that domestic animals including dogs, pigs, cattle and goats may evolve ACE2 diversity to restrict SARS-CoV2 infections.

Determining the Prevalence of Bovine Influenza D Virus in the United States
Ruminant Diseases and Immunology Research Unit, National Animal Disease Center
Ames, Iowa

Characterization of disease potential of emerging viral pathogens is important to maintain the safety and efficiency of our domestic livestock herds. Influenza D virus (IDV) is a recently characterized viral pathogen of cattle with some potential for transmission to swine. It is found by veterinary diagnostic laboratories most commonly associated with other viral pathogens. It is not clear how widespread this virus is and where it is found in the United States. A survey was conducted by ARS scientists at the National Animal Disease Center in Ames, Iowa, using cattle sera that was collected from 1,992 animals across the country in 2014 and 2015. Overall, there was a 77.5 percent positive rate for IDV in the tested sera, with regional rates varying between 47.4 and 84.6 percent. Antibody positive samples were found in 41 of 42 states from which samples were obtained. This high antibody positive rate shows that there is a need for studies to determine severity of disease caused by IDV as well as how the virus is spreading and its potential to cause disease in both cattle and swine.

Effect of Environmental Temperature on the Ability of Mosquitoes to Transmit Rift Valley Fever Virus
Arthropod-Borne Animal Diseases Research Unit
Manhattan, Kansas

ARS researchers in Manhattan, Kansas, and collaborators at U.S. Army Medical Research Institute for Infectious Diseases evaluated how environmental temperature affects the ability of two mosquito species native to the United States to transmit Rift Valley fever virus (RVFV). Incubation temperatures ranging from 14ºC to 26ºC were evaluated for their effect on infection, dissemination, and transmission rates. For both mosquito species tested, increased temperature was associated with more rapid and more efficient development of a disseminated infection, and thus increased potential for transmission of RVFV. Although environmental temperature affected the ability of RVFV to replicate and amplify, the effect differed between the two species. Thus, the results indicated that increased dissemination and transmission due to temperature was species dependent. These data on the effects of environmental factors, such as ambient temperature, ensures the accurate development of models to assess RVFV persistence and spread in nature should a disease outbreak ever occur in the United States.

Venereal Transmission of Vesicular Stomatitis Virus in Biting Midges
Arthropod-Borne Animal Diseases Research Unit
Manhattan, Kansas

Biting midges are well-known agricultural pests and are able to transmit vesicular stomatitis virus (VSV) to cattle, horses, and swine. Vesicular stomatitis outbreaks occur every 3-8 years in the United States and result in significant economic losses due to animal health, animal movement restrictions, and quarantines. In temperate regions, viruses can overwinter in the absence of infected animals through unknown mechanisms, resulting in new infections the following year. To better understand whether virus may be maintained in insect populations in these multi-year outbreaks, ARS scientists at the Arthropod-Borne Animal Disease Research Unit collaborated with Kansas State University to examine whether VSV could pass between male and female midges during mating. What they found is that VSV-infected females could transmit virus to uninfected naïve males, and infected males could transmit virus to uninfected naïve females. This research shows the importance of males in VSV transmission dynamics, and the role vectors may play in the maintenance of VSV. This is the first evidence for venereal transmission of any arbovirus in biting midges, and the first evidence for venereal transmission of VSV in any known vector species. These results highlight the need to incorporate alternative routes of transmission in understanding arbovirus outbreaks, and could lead to a more comprehensive understanding of: 1) potential virus persistence in nature between outbreaks; 2) the ability of some virus strains to survive through the winter leading to multi-year outbreaks; and 3) virus transmission dynamics during VSV outbreaks.

Component 2: Antimicrobial Resistance

Problem Statement 2B: Alternatives to Antibiotics

Mode of Action of Avian Poultry Defensin Molecules
Animal Biosciences and Biotechnology Laboratory, Beltsville Agricultural Research Center, Beltsville, Maryland

Antimicrobial peptides (AMPs) protect mammalian hosts from pathogens as a first line of defense. Defensins such as ß-defensin are a type of AMPs that have been shown to have antimicrobial activity and immune modulation effects. Avian species are also known to express ß-defensin that have antimicrobial properties against many pathogenic bacteria and fungi. The mechanism of action of avian ß-defensins is thought to be similar to those of mammalian AMPs and involves the disruption of bacterial cell membrane. ARS scientists at the Beltsville Agricultural Research Center identified a new chicken defensin called “Avian Beta Defensin 5” (AvBD5) and showed that it induced cytokines that modulated the innate immune response. The discovery of new defensins that have immune modulation effects could potentially be developed as alternatives to antibiotics to treat bacterial infections.

Dietary Allium hookeri (garlic chives) Changes the Gut Microbiome in Chickens
Animal Biosciences and Biotechnology Laboratory, Beltsville Agricultural Research Center
Beltsville, Maryland

The potential beneficial health effects of dietary *Allium hookeri* have yet to be determined but may enhance gut health by reducing oxidative stress. To better understand the mechanism of action of dietary *A. hookeri*, ARS scientists at the Beltsville Agricultural Research Center collaborated with scientists in South Korea to investigate the impacts of dietary *A. hookeri* on the gut microbiome using a nucleic acid sequencing technology to identify bacteria using samples obtained from the cecum of chickens fed with *A. hookeri* leaf. The results from this study showed that the microbiome composition in the groups supplemented with *A. hookeri* leaf had higher proportion of beneficial bacteria compared to the non-treated control group. Modulation of gut microbiome by the *A. hookeri* leaf correlated with growth traits including increased body weight, and bone strength. The conclusion from this study is that an *A. hookeri* supplemented diet is beneficial for gut health of broiler chickens via mediation of the gut microbiome.

Component 3: Zoonotic Bacterial Diseases

Problem Statement 3A: Brucellosis

Performance of a Brucellosis Diagnostic Test in Domestic and Wildlife Species
Infectious Bacterial Disease Research Unit, National Animal Disease Center
Ames, Iowa

Billions of dollars have been invested at the state and federal levels to support the eradication of Brucellosis from the United States. Brucellosis is a bacterial disease that is largely controlled in domesticated livestock but has proven to be very difficult to eradicate because of reinfection from wildlife reservoirs such as bison, elk and feral swine. It is a zoonotic disease that can spread from cattle to people and cause severe human illness. The fluorescence polarization assay (FPA) is a highly sensitive and specific test that is commonly used for brucellosis surveillance under field conditions. Although designed for use in cattle the test is also used for surveillance in elk, swine, and bison using negative control cattle sera included in the kit. To determine if species differences may influence test response, ARS scientists in Ames, Iowa, tested samples from non-infected swine, bison and elk against samples from control, vaccinated, and Brucella-infected animals. The data demonstrate that the assay results are influenced by the species. Some species' samples were incorrectly interpreted as positive for brucellosis. Additionally, the assay does not perform as well in swine and did not detect animals persistently infected with the *Brucella abortus* strain RB51 vaccine. This work will inform and caution regulatory personnel in states with brucellosis-infected wildlife, those conducting surveillance studies, and livestock producers.

Problem Statement 3C: Tuberculosis

Validation of an International Standard PPD (purified protein derivative) for Skin Testing Cattle

Infectious Bacterial Disease Research Unit, National Animal Disease Center
Ames, Iowa

Bovine tuberculosis, caused by the bacterium *Mycobacterium bovis*, is a global problem impacting international and domestic trade. Harmonization and acceptance of diagnostic tests for bovine tuberculosis are important production and trade issues. Working internationally with other bovine tuberculosis research labs and the World Organization for Animal Health (OIE), ARS scientists in Ames, Iowa, evaluated and validated a new international standard tuberculin, a sterile protein extract of *M. bovis* that is used for skin testing cattle for bovine tuberculosis. This new standard creates a global organized system to ensure uniform testing worldwide. These findings will benefit regulatory agencies, veterinarians and livestock producers involved with maintaining the tuberculosis-free status for the United States.

Component 4: Respiratory Diseases

Problem Statement 4A: Bovine

Characterization of a New strain of Bovine Viral Diarrhea Virus Isolated in the United States

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

A bovine viral diarrhea virus (BVDV) strain was isolated in California that was shown to be a BVDV1 species but was not of the BVDV1a or BVDV1b groups that are known to be in the United States. Characterization of this virus is important to maintain vigilance for emerging pestiviruses and to determine the effectiveness of current bovine vaccines in protecting against these viruses. ARS scientists in Ames, Iowa, in collaboration with researchers at the University of California, Davis, revealed that this virus was a novel BVDV1 strain that had not been previously reported in the United States. Genetic analysis of the virus sequence showed that this virus was a BVDV1i virus that had only been reported in Europe and South America. Further, tests were conducted using antibodies raised against BVDV strains that are found in commercial bovine vaccines. This showed that these antibodies recognized the BVDV1i strain but at a reduced level and suggests that current vaccines may only provide partial protection against emerging BVDV1.

A Reference Typing Method and Related Public Database for Mycoplasma bovis
Ruminant Diseases and Immunology Research Unit, National Animal Disease Center
Ames, Iowa

Mycoplasma bovis is a bacterial pathogen that causes significant respiratory disease in cattle and bison. An objective, standardized, and discriminatory method to categorize bacterial isolates is needed to understand how this bacterium spreads and whether particular families of isolates have an enhanced ability to cause disease. Two different genetic typing methods have recently been developed for *M. bovis*, but the related data available fail to reveal whether one typing method is superior to the other. To resolve this issue, a subcommittee of the International Organization for Mycoplasmology requested ARS researchers in Ames, Iowa, to organize and lead a multinational effort to compare the two methods and identify a single approach as a universal typing scheme. ARS scientists defined a single, highly informative method employed as the reference typing scheme for *M. bovis* using bioinformatics tools to analyze genome sequences from more than 450 isolates obtained from every major region of the world. The scheme and a related open-access, curated database are freely available online at pubmlst.org/mbovis. The database integrates genetic data with isolate-specific information, such as geographic and anatomic origin, year of origin, clinical presentation of the animal of origin, and other factors. This comprehensive resource currently includes more than 1,200 isolates and has been accessed by more than 100 animal health researchers and clinicians around the world. Information from the database was a critical part of several recent studies that defined local, regional, and global transmission patterns of *M. bovis*. Such insights into the population structure and epidemiology of *M. bovis* will support the development of rational, data-driven management and treatment practices that will positively impact livestock farmers and consumers of related products.

A Computational Method to Quantify the Effects of Genome Replication Errors on Bacterial Evolution
Genetics, Breeding, and Animal Health Research Unit, U.S. Meat Animal Research Center
Clay Center, Nebraska

Bovine respiratory disease costs the industry hundreds of millions of dollars each year through treatment costs and losses in production. Bovine respiratory disease is often caused by a complex of viruses and bacteria. Researchers do not understand how some bacteria normally found in the respiratory tract can suddenly become virulent. ARS researchers at Clay Center, Nebraska, developed a novel method to study how bacterial respiratory pathogens such as *Mannhemia haemolytica*, *Histophilus somni*, and *Bibersteinia trehalosi* suddenly become virulent. These bacteria all possess genomic features consisting of a single base or multiple bases, repeated in series, called simple sequence repeats or SSRs. These SSRs allow for the bacterial genes to change rapidly through mispairing of DNA polymerase. Sometimes the change is great and changes the appearance, virulence or pathogenicity of the bacterium and is referred to as phase variation. The development of methodology to track these replication errors will greatly

increase researcher's ability to understand how different bacteria cause disease and to develop mitigation strategies.

Problem Statement 4B: Porcine

Detection of Live Attenuated Influenza Vaccine Virus Circulating in the United States Swine Population

Virus and Prion Diseases Research Unit, National Animal Disease Center
Ames, Iowa

Influenza A virus (IAV) is an important respiratory disease of swine that can cause significant economic losses for producers. The genome of IAV contains 8 gene segments that can be mixed when an individual is infected with more than one strain of IAV. This process, called reassortment, results in new gene combinations in the progeny viruses that may escape the immune response induced from prior vaccination. Inactivated and live attenuated influenza virus vaccines (LAIV) are available in the United States for use in swine. ARS scientists at the National Animal Disease Center conducted a study of the genetic makeup of IAV that were detected in swine diagnostic submissions in the United States during 2018. What they found were gene combinations in viruses that were generated by reassortment events between circulating swine IAV and the strains included in the LAIV. These reassorted viral genomes represent unique viruses compared to other recently circulating IAV, which may not be recognized by the immune system of pigs previously infected by the prevalent contemporary strains circulating in the United States. The reassortment between the LAIV strains and contemporary IAV increases the genetic diversity among swine IAV, with potential impact to the swine industry if these novel IAV strains spread to non-LAIV vaccinated herds. These findings are important for informed vaccine selection for swine herds and contribute to the understanding of swine influenza evolution for animal and public health.

Genomic Regions of Streptococcus suis Reveal Differences that May Contribute to the Spectrum of Clinical Disease

Virus and Prion Diseases Research Unit, National Animal Disease Center
Ames, Iowa

Different bacterial isolates of *Streptococcus suis* cause a spectrum of disease in pigs which complicates studies to understand how the bacterium causes disease. ARS researchers at Ames, Iowa, evaluated the ability of nine isolates of *S. suis* to cause disease following intranasal challenge in swine followed by comparative genomic analyses to identify genetic differences in the isolates that could be associated with swine-virulence. Outcomes of intranasal challenge with the isolates ranged from lethal systemic disease to no signs of disease. Whole genome sequencing followed by comparative genomic analyses revealed several notable regions of difference, including regions encoding secreted and membrane-associated factors, which likely contributed to the spectrum of clinical disease observed. In addition, transmissible elements containing antimicrobial resistance genes were identified within the *S. suis* genomes. Collectively, these results provide a foundation for understanding the genomic attributes responsible for the

spectrum of virulence that exist among *S. suis* isolates. This information is paramount to designing effective vaccines needed by the swine industry to mitigate *S. suis* disease and decrease public health concerns.

Problem Statement 4B: Sheep

The First U.S. National Survey of Mycoplasma ovis in Domestic Sheep Operations
Animal Disease Research Unit
Pullman, Washington

Mycoplasma ovis is a blood pathogen in sheep, goats, and deer. While its presence has been documented in the United States, there had been no large-scale study documenting its prevalence and geographic range among sheep. ARS researchers in Pullman, Washington, in collaboration with researchers at USDA Animal and Plant Health Inspection Service performed the first national survey of *M. ovis* in U.S. sheep. Samples were collected from more than 34,000 U.S. sheep as part of the National Animal Health Monitoring Surveys. *M. ovis* was present in the great majority of U.S. sheep operations (73.3 percent). Furthermore, when present, *M. ovis* occurred in 23.3 percent of sampled individuals within the flock. These data demonstrate *M. ovis* is more common than previously believed in the United States and may contribute to anemia, jaundice, and ill-thrift (failure to thrive) for many sheep.

Component 5: Priority Production Diseases

Problem Statement 5A: Johne's Disease

Completed Genome of the Bacterium Causing Johne's Disease
Infectious Bacterial Disease Research Unit, National Animal Disease Center
Ames, Iowa

Johne's Disease is a chronic progressive intestinal disease caused by the bacterium Mycobacterium paratuberculosis (MAP) and is characterized clinically by chronic or intermittent diarrhea, emaciation, and death. Dairy herds infected with Johne's disease have significant economic losses due to reduced milk production and premature culling. The type III strain of MAP is found in sheep and goats and has characteristics of being more fastidious and difficult to culture than other MAP strains. It is also phylogenetically different. ARS scientists in Ames, Iowa, completed the genomic sequence of a MAP type III strain and published it for public use. The genomes of type I and type II strains of MAP were previously published. These data advance our understanding of genetic diversity among all MAP subtypes and contribute to an understanding of how genetic differences influence virulence. This information on MAP genetics will be of interest to producers, veterinarians, and academic scientists interested in understanding MAP virulence and identifying intervention strategies to reduce infection.

Component 6: Parasitic Diseases

Problem Statement 6B: Hemoparasitic Diseases

Vaccines for Anaplasma marginale
Animal Disease Research Unit
Pullman, Washington

Anaplasma marginale outer membrane protein vaccine candidates, OmpA Omp7, Omp8, Omp9, Am779, have high sequence and antigenic conservation in geographically distinct cattle populations. Bovine anaplasmosis, caused by the tick-borne pathogen *A. marginale*, is a production-limiting disease of cattle with a worldwide distribution and an estimated cost of $10 to 30 million annually in the United States alone. Vaccine development is hampered by variation between strains and the need for a cross-protective vaccine. OmpA, Omp7, Omp8, Omp9, Am779 are all high priority vaccine candidates. ARS researchers in Pullman, Washington, in collaboration with colleagues at the University of Ghana and the University of Pretoria in South Africa have determined: 1) That all of these proteins are highly conserved in *A. marginale* strains from west and south Africa; and 2) Antibody in animals protectively immunized with a North American *A. marginale* strain readily recognize the corresponding proteins in African strains. These findings indicate that a single multi-valent vaccine has the potential to protect against multiple strains of *A. marginale*.

Component 7: Transmissible Spongiform Encephalopathies

Problem Statement 7B: Genetics of Prion Disease Susceptibility

Discovery of a Novel Strain of Chronic Wasting Disease Present in Experimentally Inoculated LL132 elk
Virus and Prion Research Unit, National Animal Disease Center
Ames, Iowa

Chronic wasting disease (CWD) is a fatal disease of deer and elk that causes damaging changes in the brain. The infectious agent is an abnormal protein called a prion that has misfolded from its normal state. Whether or not an elk will get CWD is affected by their genetics. Scientists at the National Animal Disease Center evaluated the transmission of abnormal prion protein from elk of three different genotypes that were infected with CWD to transgenic mice expressing the elk prion protein. Previous work by ARS scientists demonstrated that there are differences in incubation periods, patterns of abnormal prion accumulation in the brain, and their stability in these different genotypes of elk. This study demonstrated that elk donor genotype-associated differences in relative incubation periods, stability, and lesions in the brain were maintained across first and second passages to mice suggesting that they are different CWD strains that may require different approaches for prevention and eradication. This information may be useful to

wildlife managers and captive wildlife owners that are selectively breeding animals and could impact future regulations for the control of CWD in the United States.

Problem Statement 7C: Diagnostics, Detection, and Prevention

Development of an Improved Enrichment Method for Amplification-based Prion Detection

Virus and Prion Diseases Research Unit, National Animal Disease Center
Ames, Iowa

Prion diseases are fatal neurodegenerative diseases that affect a wide range of livestock and wildlife. The disease process occurs through the misfolding of a normally occurring protein. Detection of this misfolded protein is the only known means by which a prion disease can be diagnosed. A recently developed approach for the detection of this misfolded protein uses a technique referred to as Real-time quaking induced conversion (RT-QulC). RT-QulC amplifies the amount of misfolded protein available for detection but can be inhibited by naturally occurring contaminants in the tissue samples used for the technique. ARS scientists at the National Animal Disease Center successfully developed a new method to clean the samples prior to analysis, demonstrating the cleanup technique enhanced the detection and reduced the time required to assess the results providing additional tools for diagnostic laboratories.