Animal Health (NP 103) Annual Report for 2022

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 significant contributions to the agricultural economy. A 2017 National Agriculture Statistics Service (NASS) Census of Agriculture Report identified the U.S. livestock industries produced over $138 billion in farm gate receipts across all major food animal producing species. There are currently 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, while the poultry industry produces $42.7 billion and 5.4 million small ruminants produce $844 million. However, animal disease outbreaks continue to result in production losses and economic damages to producers. Furthermore these losses have ripple effects into other parts of the economy that are dependent upon the livestock industry for production of goods or that serve the livestock industries such as advanced genetics, veterinary providers, and animal feed industries. Importantly, foreign animal diseases and the emergence of new pathogens continue to pose a threat to our livestock industries.

From a One Health perspective, protecting animals and public health also means protecting against zoonotic diseases. Zoonotic diseases may be endemic and already occur in the United States, but many are foreign animal diseases that pose a significant threat if they were to be introduced into the country. In either case, it is critically important that new and innovative tools such as diagnostics and vaccines are developed for early detection, control, and where feasible, eradication of these diseases. ARS conducts basic and applied research in the following research areas to deliver these solutions:

1. Biodefense
2. Antimicrobial Resistance
4. Endemic Bacterial Diseases
5. Endemic Viral Diseases
6. Parasitic Diseases
7. Transmissible Spongiform Encephalopathies

In Fiscal Year (FY) 2022, NP 103 researchers continued to conduct emergency response research to address continued concerns with highly pathogenic avian influenza, and potential SARS-CoV-2 spill overs between humans and animals.

Notable in 2022 was the continued spread of endemic diseases such as Chronic Wasting Disease in white tailed deer. Amongst the myriad of foreign animal diseases, African swine fever (ASF) stood out with its continued spread throughout Asia, leading to the culling of millions of pigs. While ASF has not reached the United States, it was recently found in the Dominican Republic and Haiti, increasing the threat for U.S. pork producers.

In 2022, the Animal Health National Program initiated the 1st year of a new 5-year national program cycle, during which significant accomplishments were achieved 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 44 core research projects, with the support of 131 (including vacancies) scientists located at eleven research sites throughout the United States. The FY2022 ARS research budget for the Animal Health Program was $91.2 million with increases for the science program at the National Bio and Agro-defense Facility. Scientists working in the program published 179 manuscripts in peer-reviewed journals.

Significant technology transfers were achieved with:

- 11 new inventions disclosures;
- The submission of 5 patent applications,
- 8 licenses issued for ARS patents;
- The establishment of 9 research agreements; and
- 4 new patents awarded.

The NP103 program also trained 54 students and post-doctoral candidates during FY2022.

New additions to the NP 103 team in 2022 are:

Dr. Karen Poh, Research Entomologist, joined the Animal Disease Research Unit, Pullman, WA.

Kennan Oyen, Research Entomologist, joined the Animal Disease Research Unit, Pullman, WA.

Dr. Stacey Scroggs, Research Microbiologist, joined the Arthropod-Borne Animal Disease Research Unit, Manhattan, Kansas.

Dr. Bryan Kaplan, Research Microbiologist, joined the Ruminant Diseases and Immunology Research Unit, Ames, Iowa.

The following scientists in NP 103 received prominent awards in 2022:

Dr. Joan Lunney was awarded the Presidential Rank Meritorious Senior Professional Award for sustained accomplishments.

Dr. Dana Nayduch received the Lifetime Achievement Award for recognition of her outstanding contributions to animal health and productivity, her vocal advocacy for the profession of livestock entomology, and her active engagement in the organization.

Dr. Jonathan Artz received the Dr. Daniel E. Salmon award for recognition of his distinguished service, exemplary leadership, and pioneering contributions to the promotion of public health, infectious disease control and the management of neglected health problems.

Dr. Douglas Gladue was awarded the Arthur S. Flemming Award for recognition of performing outstanding service in the field of basic science.

Dr. Lindsay Allen and Dr. Terry Isabell were inducted into the USDA Agricultural Research Service Science Hall of Fame.

Drs. Elizabeth Rieder and Luis Rodriguez received the 2022 Excellence in Technology Transfer National Award for the development of the Foot-and-Mouth Disease (FMD)LL3B3D vaccine from the Federal Laboratory Consortium for Technology Transfer.

Drs. Manuel Borca and Doug Gladue received the 2022 Excellence in Technology Transfer National Award for the development of the African swine fever vaccine (Delta I177L) from the Federal Laboratory Consortium for Technology Transfer.

Research Results:

COVID-19 Impacts: Despite limited access to the laboratories due to COVID-19 social distancing, the NP103 scientists delivered high-impact scientific accomplishments through publications and presentations, including several studies on which livestock species are susceptible to infection with the SARS-CoV-2 virus.

The following section of the report provides examples of high impact research results that address the objectives in the current national program action plan components.

Component 1: Biodefense

Problem Statement 1A: Control and eradicate foreign animal diseases

Development and approval of the 1st African swine fever vaccine worldwide

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 were no commercial vaccines. ARS scientists at the Plum Island Animal Disease Center (PIADC) successfully developed innovative genetic engineering techniques that enabled the discovery of a live attenuated vaccine called ASFV-G-dI177L, which was shown to be fully protective and safe in experimental clinical studies at PIADC. The vaccine was subsequently transferred in June 2020 to the National Veterinary Joint Stock Company (NAVETCO) in Vietnam through a Research Agreement. ARS scientists working in partnership with NAVETCO successfully tested, in record time, the vaccine against locally circulating Vietnamese ASF virus field strains in pigs of European and Asian genetic background. NAVETCO also showed that ASFV-G-dI177L is genetically stable, remains attenuated, and lacks local and general toxicity when inoculated in domestic pigs. NAVETCO received a certificate of Marketing Authorization from the Vietnamese Department of Agriculture and Rural Development on June 3, 2022, making ASFV-G-dI177L the first ASFV vaccine ever approved for commercial use. The vaccine is currently being deployed under control field conditions in swine farms in Vietnam to further evaluate its safety and efficacy characteristics. If successful, Vietnamese authorities will develop plans to integrate ASF vaccination in their National ASF Control Program.

How new foot-and-mouth disease viruses emerge in nature.

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

Foot and mouth disease (FMD) is an easily transmitted, devastating disease of livestock, and the virus is capable of persisting without causing disease, which has important implications FMD control strategies. This phenomenon is called the “carrier state” and until now was widely perceived as a dead end because of the belief that persistently infected cattle could not transmit the virus to other animals. ARS scientists in Orient Point, New York, demonstrated that when persistently infected carriers of FMDV were exposed to a different strain of the virus, the two viruses exchanged genetic material (recombined) to give rise to new viruses containing distinct parts of each of the parental viruses. This discovery demonstrates a novel process whereby new strains of FMDV may evolve and emerge in the field and contributes towards preparing for a potential outbreak of new and emerging FMDV strains that could pose a threat to the U.S. homeland. The work was published in the journal Pathogens in 2022 (https://www.mdpi.com/2076-0817/11/6/644).

Problem Statement 1B: Predict and prevent emerging diseases Problem

White-tailed deer are susceptible to SARS-CoV-2, but cattle, poultry, and swine are not.

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

Southeast Poultry Research Laboratory
Athens, Georgia

National Animal Disease Center
Ames, Iowa

It is likely that the COVID-19 pandemic caused by the SARS-CoV-2 virus originated in bats and passed through an unknown animal host before being transmitted to humans. ARS scientists in Ames and Athens worked together to perform emergency response, high-priority research to determine the susceptibility of various livestock species to infection with the SARS-CoV-2 virus. Their results indicated that cattle, swine, chickens, turkeys, ducks, quail, and geese were not susceptible to SARS-CoV-2. However, white-tailed deer were highly susceptible; they did not demonstrate clinical symptoms but shed large amounts of virus in the first 5-6 days after infection, and readily transmitted the virus to other deer. APHIS Wildlife Services then initiated surveys of SARS-CoV-2 in wild white-tailed deer and found it was possible for deer to act as a reservoir for the virus and transmit it to humans. ARS scientists also demonstrated that farmed mink are susceptible to SARS-CoV-2 infection and viral shedding, despite not exhibiting symptoms. This information is critical for consumers, scientists, livestock producers, and regulatory officials who have public health responsibilities.

Component 2: Antimicrobial Resistance

Problem Statement 2A: Combat antimicrobial resistance through the development of Alternatives to Antibiotics

Antibacterial activity and stability of a new antimicrobial peptide.

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

The increasing prevalence of antibiotic resistance among pathogenic microbes highlights the urgent need for the identification and development of alternatives to antibiotics. Antimicrobial peptides (AMPs) are highly effective against microbial pathogens that cause diseases in humans and animals, but they are sensitive to proteases and kidney clearance. ARS scientists in Ames, Iowa, developed a stable small peptide and tested it for resistance against degradation, stability, toxicity, and *in vitro* and *in vivo* anti-bacterial activities against *Histophilus somni*, one of the bacterial organisms causing respiratory diseases in cattle. The peptide was able to kill *H. somni* very efficiently. These results demonstrate the possible use of an alternative treatment for controlling bacteria that cause respiratory diseases in cattle.

Component 3: Endemic Bacterial Diseases

Problem Statement 3A: Mitigate the consequences of zoonotic bacterial diseases

New candidates identified for development of a better Leptospira vaccine

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

Leptospirosis is a zoonotic disease that causes reproductive losses and infertility in cattle. Vaccines to prevent Leptospirosis have limited efficacy and a short duration of immunity. One limitation in developing new vaccines is the lack of culture systems that mimic conditions in the animal. To address this knowledge gap, ARS scientists in Ames, Iowa developed and used a new media to grow *Leptospira* at temperatures encountered during host infection and identified proteins that had increased abundance at body temperature and may produce a more robust immune response in vivo. Thus, these proteins are attractive targets for development of novel vaccines. This work may be foundational for development of improved vaccines to prevent leptospirosis and will be of interest to stakeholders, regulatory personnel, and researchers with interest in leptospirosis and leptospirosis vaccines.

Statement 3B: Mitigate respiratory bacterial diseases of livestock species

Development of new mucosal vaccine to control pneumonic Pasteurellosis in cattle

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

Mannheimia haemolytica remains the largest single cause of disease losses to the cattle industry, and there is strong interest in the cattle industry for improved vaccines against pneumonic Pasteurellosis coupled with mucosal vaccine delivery. A novel vaccine against bovine pneumonic Pasteurellosis caused by *Mannheimia haemolytica* was developed and tested by ARS researchers in Ames, Iowa. A calf vaccination and challenge study demonstrated that a single intranasal dose of the vaccine confers protection against disease following challenge with virulent *Mannheimia haemolytica*. The protection was associated with major reductions in the amount of the disease agent in lungs following challenge, and reduced lung damage. The cattle industry is interested in the commercialization of this new mucosal vaccine.

Problem Statement 3C: Diagnose and mitigate strategies for production related bacterial diseases

Identified genetic variants associated with bovine congestive heart failure in feedlot cattle

Animal Health Genomics Research Unit

U.S. Meat Animal Research Center
Clay Center, Nebraska

Bovine congestive heart failure (BCHF) in feedlot cattle is increasingly common in North America's western Great Plains; reported losses in individual feedlot operations exceed $250,000 annually, which could represent 10-20 percent of a feedlot's income. While BCHF is an untreatable, complex, and fatal condition, cattle herds affected with BCHF are typically bred and managed to achieve high-quality carcasses, so reducing BCHF's impact is a priority for the beef industry. ARS researchers in Clay Center, Nebraska, evaluated animals with end-stage heart failure from 30 different ranch sources, together with their healthy pen mates. The researchers discovered DNA sequence variations (risk markers) in two major genes that is likely to play a role in BDHF development. Feedlot animals that had the risk markers were 28-fold more likely to develop heart failure than those without the markers. These markers will serve as the basis for a genetic test that can identify feedlot animals at the highest risk for BCHF in North America's western Great Plains. In herds suffering from BCHF, identifying high and low risk cattle will enable producers to make informed decisions for selective breeding and animal health management to reduce the impact of this disease.

Dairy cows not selected for high milk production respond better the mastitis challenge than modern cows
Ruminant Diseases and Immunology Research
National Animal Disease Center

Selective breeding for increased milk production may through time lead to greater health problems. ARS researchers in Ames, Iowa, showed that cows with genetics found in 1964 could clear experimental mastitis bacteria almost immediately and had fewer clinical signs of infection than modern Holsteins. Bacteria was barely detectable in the unselected cows in contrast to the modern cows, which had nearly 100,000 bacteria per milliliter of milk after 12 hours of infection. Surprisingly, the milk immune cell counts were similar in both genotypes despite the massively lower bacterial counts in the unselected cows. This suggests that 1964 cows have an innate immune system that is far more effective than that of modern Holsteins. This work is a prelude to finding the genes and mechanisms that allowed better immune competency in unselected cows and bringing those genes back into the modern dairy cow. Once the genes and mechanisms that improve immune competency are identified, it will be possible to select them in the modern Holstein, minimizing mastitis and improving milk production.

Component 4: Endemic Viral Diseases

Problem Statement 4B: Enhance the control of viral diseases in intensive production systems

Expression Profiles and Interaction of MicroRNA and Transcripts in Response to Bovine Leukemia Virus Exposure
Ruminant Diseases and Immunology Research Unit
National Animal Disease Center

Ames, Iowa

Bovine leukemia virus (BLV) infection in cattle is omnipresent, which causes significantly economical losses worldwide. The objective of this study was to determine microRNA (miRNA) and transcript profiles and to establish their relationship in response to exposure to the virus. Small noncoding and messenger RNA were extracted and sequenced from serum and white blood cells (WBCs) derived from seven BLV seropositive and seven seronegative cows. Transcriptomic profiles were generated by sequencing RNA libraries from WBC. Bta-miR-206 and bta-miR-133a-3p were differentially expressed in serum ($P < 0.05$). In WBC, bta-miR-335-3p, bta-miR-375, and bta-novel-miR76-3p were differentially expressed ($P < 0.03$). There were 64 differentially expressed transcripts (DETs). Gene ontology (GO) analysis of the DETs overexpressed in the seropositive group with GOs of response to stimulus and immune system process predicted that the DETs could potentially negatively regulate viral life cycle and viral entry or release from host cells. In addition, the DETs depleted in the seropositive group could play a role in the downregulation of antigen processing and presentation of endogenous peptide antigen *via* MHC class I. The differentially expressed miRNAs targeted 17 DETs, among which the expressions of bta-miR-133a-3p and bta-miR-335-3p were significantly negatively correlated with the expressions of ENSBTAT00000079143 and ENSBTAT00000066733, respectively. Under high prediction criteria, 90 targets of the differentially expressed miRNAs were all non-DETs. The most enriched biological process GO term of the targets was the RNA-dependent DNA biosynthetic process, which could be associated with virus replication. These results suggested that the differentially expressed miRNAs fine-tune most of the target genes in responding to BLV exposure. In addition, Bta-miR-206 interacted with BLV regulatory genes *rex* and *tax* by targeting their coding regions. A further study of the miRNAs and the genes may reveal the molecular mechanisms of BLV infection and uncover possible ways to prevent the infection.

Component 5: Parasitic Diseases

Problem Statement 5A: Improve diagnostic and mitigation strategies for gastrointestinal (GI) parasitic diseases

Improved computational tools for assessing how microbiome composition changes through time

Animal Parasitic Diseases Laboratory
Beltsville Agricultural Research Center
Beltsville, Maryland

The composition of the microbiome changes through time, confounding attempts to understand global animal responses to changes in diet, veterinary treatment, or husbandry conditions. However, irregular sampling intervals and missing data sometimes plague efforts to understand these responses. ARS scientists in Beltsville, Maryland, developed user-friendly workflows for longitudinal genomic and microbiome analyses. They compared and improved batch correction and group difference detection algorithms to better discern changes through time. These improvements enable microbiologists,

veterinarians, and livestock producers to harvest better data, from fewer animals, when seeking to understand how the microbiome changes through time.

Problem Statement 5B: Prevent spread of Hemoparasitic diseases of livestock

Theileria haneyi causes less severe disease in horses compared to Theileria equi
Animal Disease Research Unit
Pullman, Washington

Theileria haneyi and *T. equi* are tick transmitted parasites that cause the highly regulated disease equine theileriosis. *T. haneyi* was recently discovered by ARS scientists in Pullman, Washington. These scientists demonstrated that *T. haneyi* is resistant to the gold-standard anti-theilerial therapy, imidocarb dipropionate, and that it interferes with treatment of horses with *Theileria equi* in co-infected animals. However, the severity of disease caused by *T. haneyi* was not fully understood prior to this study. ARS researchers in Pullman, Washington, demonstrated that *T. haneyi* is significantly less virulent than *T. equi,* and even horses that have undergone removal of the spleen (splenectomy), an organ crucial to blood parasite control, survive infection. This is in stark contrast to *T. equi*, which is almost always fatal in splenectomized horses. Furthermore, a majority of splenectomized horses spontaneously cleared *T. haneyi* after months of persistent, asymptomatic infection. Thus, infection with *T. haneyi* alone will likely cause minimal disease in most horses. This data provides critical information on the potential impact of this disease for our U.S. equine stakeholders.

Component 6: Transmissible Spongiform Encephalopathies

Problem Statement 6B: Reveal genetics of prion disease susceptibility

The placenta of sheep with atypical scrapie is not infectious
Animal Disease Research Unit
Pullman, Washington

Scrapie is a disease of sheep caused by the accumulation of abnormal infectious proteins, called prions, in the central nervous system. Concerted efforts to breed sheep with genetic markers associated with high resistance to the classical forms of naturally transmissible scrapie prions has led to the near eradication of this disease in the United States. Nor98-like scrapie is a sporadic, atypical form of scrapie thought to spontaneously arise in the central nervous system of aging sheep. ARS researchers in Pullman, Washington, confirmed that while these atypical prion-like proteins accumulate in the placenta of scrapie-resistant sheep, the placental proteins are not infectious. This knowledge supports field evidence that Nor98-like scrapie is a spontaneous disease of sheep with low to no risk of natural transmission. Furthermore, this knowledge supports the continued use of selective breeding of scrapie resistant sheep to mitigate the occurrence of classical forms of scrapie in sheep.