Animal Health (NP103)
Annual Report for 2023

Introduction

The goal of the Animal Health National Program (NP103) is to protect and ensure the safety of the Nation's agricultural animals and economy, the food supply, and public health through research to improve 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 more than $138B 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 $50.2B[1] from cattle and calf production and an additional $38.1B in milk alone. There are 73.2 million pigs in the United States that produce $19.2B in goods, while the poultry industry produces $42.7B and 5.4 million small ruminants produce $844M. 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 producing goods or that serve the livestock industries, such as advanced genetics, veterinary providers, and animal feed industries. Foreign animal diseases and the emergence of new pathogens continue to pose a threat to our livestock industries.

Vision: The NP 103 vision is to be a worldwide leader delivering effective solutions to prevent and control animal diseases that impact agriculture and public health.

Mission: The NP103 mission is to conduct basic and applied research on selected diseases of economic importance to the United States livestock and poultry industries.

Strategic Alignment

NP103 supports the USDA Strategic Plan Goal 2, *Ensure America's Agricultural System is Equitable, Resilient, and Prosperous*, and Goal 3, *Foster an Equitable and Competitive Marketplace for All Agricultural Producers*. NP103 also supports Objective 2.2, *Protect Agricultural Health by Minimizing Major Disease, Pests, and Wildlife Conflicts*.

NP 103 also supports the USDA Science and Research Strategy Priority 1, *Accelerating Innovative Technologies & Practices*, and Priority 4, *Cultivating Resilient Ecosystems*.

From a One Health perspective, protecting animals and public health also means protecting against zoonotic diseases (infections that are spread between animals and people). 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 are introduced into the country. In either case, it is critically important that new and innovative tools such as

[1] Source: https://www.ers.usda.gov/topics/animal-products/cattle-beef/statistics-information/

diagnostics and vaccines are developed for early detection, control, and where feasible, eradication of these diseases worldwide. ARS conducts basic and applied research in the following research areas to deliver these solutions:

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

2023 Accomplishments

In Fiscal Year (FY) 2023, NP103 researchers continued to conduct emergency response research to address continued concerns with new disease introductions in the United States, such as highly pathogenic avian influenza, and potential SARS-CoV-2 spill overs between humans and animals.

Notable in 2023 was the continued spread of endemic diseases such as chronic wasting disease in white-tailed deer. Among many 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 2023, NP103 started the second year of a 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.

Dr. Cyril Gerard Gay and Dr. Roxann Motroni lead NP103, which currently includes 44 core research projects, with the support of 138 (including vacancies) scientists located at 9 research sites throughout the United States. The FY 2023 ARS research budget for NP103 was $123M with increases for the science program at the National Bio and Agro-defense Facility. Scientists working in the program published 175 manuscripts in peer-reviewed journals.

Significant technology transfers were achieved with:

- **Nine new inventions disclosures;**
- **Three patent applications submitted;**
- **Twenty-four licenses issued for ARS patents;**
- **Fifteen research agreements established; and**
- **Three new patents awarded.**

The NP103 program also trained 67 students and post-doctoral candidates during FY 2023.

New additions to the NP103 team in 2023:

- **Dr. Kaitlyn Sarlo-Davila, Dr. David Holthause, and Dr. Daniel Nielsen** joined the Ruminant Diseases and Immunology Research Unit, Ames, Iowa.
- **Dr. Meghan Wymore Brand** joined the Virus Prion Research Unit, Ames, Iowa.

- **Dr. Jimmy Pitzer, Dr. Amy Hudson, and Dr. David Molik** joined the Arthropod-Borne Animal Disease Research Unit, Manhattan, Kansas.

The following scientists in NP103 received prominent awards in 2023:

- **Dr. Joan Lunney** received the Distinguished Veterinary Immunologist Award for achievements in science and immunological toolkits, in in-field innovation, and mentoring new generations of veterinary immunologists.
- **Dr. Justin Greenlee** was awarded the Mid-Career Excellence Award for Novel Discoveries in Prion Research.
- **Dr. David Suarez** received the United States Animal Health Association Federal Partnership Award.

The following scientists retired in 2023:

Dr. David Swayne, Laboratory Director, Southeast Poultry Research Laboratory, Athens, Georgia.

Research Results:

This 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.

The FlagT4G vaccine confers a strong immunity and early virological protection against classical swine fever.

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

Controlling classical swine fever virus (CSFV) in endemic countries relies on rapid interventions to prevent the spread of the virus on infected farms, so CSFV vaccines need to quickly induce a protective immune response to protect pigs. ARS scientists in Orient Point, New York, previously showed that the FlagT4G vaccine they developed could protect pigs within a few days, but the mechanism that conferred this rapid protection was unknown. ARS scientists assessed the immune response elicited by the FlagT4G vaccine to understand its ability to protect pigs quickly. Animals were given a single dose of the FlagT4G and then challenged with a highly virulent CSFV strain 5 days later. The vaccines induced significant levels of proteins associated with early immune responses, such as interferons, and vaccinated animals showed clinical and virological protection in the absence of any antibody response. All vaccinated pigs then challenged with a virulent strain of CSFV rapidly produced CSFV neutralizing antibodies and interferon-γ producing cells. Unvaccinated pigs that were challenged showed severe clinical signs and high viral replication and were unable to generate neutralizing antibodies and an interferon-γ responses. Results show the fast and efficient protection of the FlagT4G vaccine is associated with an increase in neutralizing antibodies and an interferon-γ response, a potentially new promising tool for CSFV control worldwide.

Novel recombinant foot-and-mouth disease viruses arise within the first 48 hours of infection of persistently infected carrier cattle.

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

ARS researchers in Orient Point, New York, previously documented that foot-and-mouth disease viruses (FMDV) can cause a prolonged subclinical infection in the upper respiratory tract of infected cattle. ARS scientists further demonstrated that exposing these FMDV carriers to a different variant of the virus gives rise to novel recombinant virus variants with distinct components derived from each of the two infecting viruses. Recent results demonstrated that these recombinant viruses arise in specialized cells of the upper respiratory mucosa within the first 48 hours of infection. This finding confirms that the recombinant viruses are present in the respiratory tract during the very early stages of infection, when infected animals are known to shed the greatest amounts of virus. This discovery highlights the importance of detection and appropriate management of persistently infected FMDV carriers following disease outbreaks, especially in regions where multiple FMDV variants are in circulation.

Problem Statement 1B: Predict and prevent emerging diseases.

The diversity and evolution of influenza A virus in pigs is linked to the emergence of new influenza viruses with potential to infect humans.

Southeast Poultry Research Laboratory, Athens, Georgia

Human-to-swine transmission of the 2009 H1N1 pandemic influenza virus repeatedly occurred in the past decade and increased the genetic diversity of the virus in pigs. ARS scientists in Ames, Iowa, and Iowa State University collaborators measured the frequency of human-to-swine transmissions of the H1N1 pandemic influenza virus between 2009 and 2021 and determined how this affected the genetic diversity of these influenza viruses in swine and their potential risk of transmission to people. The scientists detected 370 separate human-to-swine transmissions when infections were the highest in the human population. Most spillovers were single events without sustained transmission, but a small subset resulted in the emergence, persistence, and co-circulation of new strains of H1N1 pandemic influenza viruses in U.S. pigs. All new strains of H1N1 pandemic influenza viruses that evolved from pigs were genetically different from human seasonal vaccine strains. These results suggest that the swine industry could reduce the transmission of influenza viruses from livestock caretakers into pigs through appropriate biosecurity measures and effective vaccination strategies, which would reduce the resulting genetic diversity of influenza viruses in pigs, and proactively reduce the potential for future swine-to-human transmission of new strains with pandemic potential.

Component 2: Antimicrobial Resistance

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

First gene-edited calf with resistance to common cattle virus

Animal Health Genomics Research Unit, U.S. Meat Animal Research Center, Clay Center, Nebraska.

Bovine viral diarrhea virus (BVDV) is one of the most widespread and economically important viral infections in cattle, with annual losses approaching $1B in the United States alone. The BVD virus infects cattle through a receptor on the cattle's cells. ARS researchers at Clay Center, Nebraska, showed that a small genome edit modifying only six amino acids in the receptor caused a dramatic reduction in BVDV susceptibility in a calf with no adverse effects in the first two years of life. This provides the first example of gene editing in cattle to reduce the impact of a major viral disease. This approach could significantly improve animal welfare, increase the long-term sustainability of cattle production, and, because BVDV infection puts calves at risk for secondary bacterial diseases, provides an opportunity to reduce antibiotic use in agriculture.

Diagnostic test kits for the early detection of necrotic enteritis infection in commercial broiler chickens.

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

Necrotic enteritis is a bacterial disease caused by Gram-positive toxicogenic *Clostridium perfringens* and costs the global poultry industry more than $6B annually. The antibiotic-free production of commercial poultry has been associated with the increasing incidence of necrotic enteritis, and many commercial poultry production facilities are becoming more dependent on using antibiotic-alternative feed additives. ARS scientists in Beltsville, Maryland, and University of Georgia collaborators developed diagnostic test kits to detect *Clostridium perfringens* proteins, which are major virulence factors involved in necrotic enteritis pathogenesis, for early detection of outbreaks in commercial broiler farms. These test kits showed that pathogenic strains of *Clostridium perfringens* can be identified in fecal samples from commercial farms within 1-2 weeks post-hatch, enabling producers to implement timely interventions to control necrotic enteritis outbreaks.

Component 3: Endemic Bacterial Diseases

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

Identified a novel tuberculosis vaccine oral delivery system for use in white-tailed deer.

Infectious Bacterial Diseases Research Unit, National Animal Disease Center,

Ames, Iowa

Tuberculosis is a chronic disease that can be transmitted to humans from animal reservoirs. Wildlife reservoirs of tuberculosis are a challenging obstacle to eradication of this disease in the United States. In Michigan, prevalence of disease in wild deer populations has not changed for 2 decades and approximately 2-3 cattle herds are infected annually. Depopulation of infected cattle herds causes significant economic costs for producers. New intervention strategies, such as vaccines, are needed to prevent tuberculosis transmission from infected wild deer. Using the "BCG" tuberculosis vaccine, ARS scientists in Ames, Iowa, demonstrated the efficacy of oral delivery of liquid BCG

to white-tailed deer. To address issues associated with delivery of liquid vaccines to wild deer, ARS scientists collaborated with USDA-APHIS scientists to develop small edible alginate spheres containing liquid BCG. Immune responses in deer eating alginate sphere vaccines were equal to those of deer receiving orally delivered liquid BCG, demonstrating this oral platform can be used to develop edible baits for vaccinating wild deer and is effective in reducing tuberculosis transmission between deer and from deer to cattle. A tuberculosis vaccine for deer would be of interest to regulatory personnel, livestock producers, and wildlife health officials.

Problem Statement 3B: Mitigate respiratory bacterial diseases of livestock species.

Identification of protein targets for a *Mycoplasma bovis* vaccine.

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

M. bovis causes respiratory disease in cattle and bison. In cattle, *M. bovis* interacts with other pathogens as part of a polymicrobial complex involved with disease. In bison, *M. bovis* is a primary pathogen that causes pneumonia with high fatality rates. Infections in bison are so problematic to treat successfully that treatments are not recommended, so there is a need for vaccines to control *M. bovis* in both cattle and bison. ARS researchers in Clay Center, Nebraska, analyzed the genomes of 240 *M. bovis* strains isolated from either cattle or bison and analyzed the vaccine potential of their proteins. They focused on proteins that either reside on the outer membrane of *M. bovis* or are secreted, as both types can work effectively in a vaccine. Regions of genetic diversity and immunological potentials were mapped for each protein of interest. This work provides a novel reference for the research community to use to design vaccines that could protect both cattle and bison from *M. bovis* and reduce devasting losses of bison to this pathogen.

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

Novel assay for *Leptospira* identification.

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

Leptospirosis is a serious zoonotic disease found at the environment-wildlife-livestock interfaces that continues to pose a threat to animals and humans. In humans and animals, the disease presentation ranges from malaise to kidney and liver failure and death. In humans, the estimated global productivity cost of leptospirosis in 2019 was $ 29.3 billion due to the diseases it causes. In collaboration with academic scientists, ARS scientists in Ames, Iowa, validated a culture-independent DNA capture and enrichment system for characterizing *Leptospira* genomic data from field samples. The assay greatly increases *Leptospira* recovery DNA from field samples and facilitates robust species identification and high-resolution genotyping. Recovered DNA sequence data is similar in quality to data from in vitro cultured isolates. Implementating this DNA capture and enrichment assay improves identifying *Leptospira* in unculturable samples and expands *Leptospira* populations and genomic diversity knowledge and understanding. The assay will improve epidemiologic knowledge of Leptospira distribution, facilitate improved diagnostics and vaccines development, and will interest diagnostic personnel, scientists, and livestock

producers. The assay already demonstrated that U.S. cattle can be infected with more than one species of Leptospira concurrently.

Component 5: Parasitic Diseases

Problem Statement 5B: Prevent spread of hemoparasitic diseases of livestock.

***Rhipicephalus microplus* and *Dermacentor variabilis* ticks are not able to transmit the U.S. isolate of *Theileria orientalis* Ikeda.**

Animal Disease Research Unit, Pullman, Washington

The tick-transmitted blood parasite *Theileria orientalis* Ikeda recently emerged as a novel threat to the U.S. cattle industry. Its emergence occurred in concert with the U.S. invasion of its primary tick vector, *Haemaphysalis longicornis* in 2017, and infected cattle herds were detected in multiple states. It is not yet known whether other tick species native to the United States and surrounding countries are also capable of transmitting *T. orientalis*. Since different tick species live in defined host geographic ranges, determining which tick species can transmit *T. orientalis* will enable predicting where the parasite is most likely to spread in the United States, thereby improving *T. orientalis* control. Using controlled studies, ARS scientists in Pullman, Washington, determined that *Rhipicephalus microplus* and *Dermacentor variabilis* ticks are unable to transmit *T. orientalis* to cattle, which provides important information for cattle producers, veterinarians, and regulatory personnel.

Component 6: Transmissible Spongiform Encephalopathies

Sheep scrapie agent can infect white-tailed deer after oronasal exposure.

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

The origin of chronic wasting disease (CWD) is not known, but it has many similarities to the sheep prion disease called scrapie. It has long been hypothesized that CWD arose through transmission of sheep scrapie to deer. ARS researchers in Ames, Iowa, conducted research to determine if scrapie derived from sheep could be transmitted to white-tailed deer. The deer inoculated with sheep scrapie developed clinical signs and the abnormal prion protein could be detected in a wide range of tissues. These results indicate that deer may be susceptible to sheep scrapie if exposed to the disease in natural or agricultural settings. In addition, several strong similarities between CWD in white-tailed deer and the experimental cases of scrapie in white-tailed deer suggests that it would be difficult to distinguish scrapie from CWD in deer or identify scrapie if a case occurs. This information should be considered by deer farmers for keeping their herds free from prion diseases.