Animal Health (NP 103) Annual Report for 2021

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 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, economic damages to producers, and can have ripple effects into other parts of the economy that are dependent upon the livestock industry for production of goods or 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 are developed for early detection, and vaccines to control, and where feasible, eradicate 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) 2021, NP 103 researchers continued to conduct emergency response research to address concerns that SARS-CoV-2 can spill over from humans to animals. One important discovery reported by ARS scientists was the first report that white tail deer were susceptible to SARS-CoV-2 and could rapidly spread the virus to other deer.

Meanwhile, there continued to be notable foreign animal disease outbreaks worldwide, including the continued spread of endemic diseases such as Chronic Wasting Disease. Amongst the myriad of foreign animal diseases, African swine fever (ASF) continued to stand 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 risk for U.S. pork producers. Although no licensed vaccine for this disease currently exists, ARS scientists have successfully transferred new vaccine candidates to industry partners for further research and development and continue to share their expertise globally[1,2].

Additionally, in FY2021, USDA kicked off the first-ever “Mitigating Zoonotic Threats” Scialog[3] as part of the National Bio and Agro-defense Facility (NBAF) Agrosecurity Partnerships for Innovative Research (ASPIRE) Program. The Scialog or “Science Dialogue” brings together early-career scientists from multiple disciplines to generate an interdisciplinary community through three annual meetings focused on finding innovative solutions.

Two new research units were created within NBAF, the Foreign Arthropod Borne Animal Disease Research Unit (FABADRU) and the Zoonotic and Emerging Disease Research Unit (ZEDRU).

A “Detect to Protect” Chronic Wasting Disease (CWD) crowd-sourcing challenge was launched FY2021 for the early detection of CWD in live animals, prior to clinical signs. To date six contestants were awarded prizes in Phase 1 of the project and selected to move to Phase 2 of the challenge.

In 2021, the Animal Health National program completed the 5th year of the 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 41 core research projects, with the support of 122 (including vacancies) scientists located at nine research sites

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[1] https://www.ars.usda.gov/GARA/events.htm

[2] https://vietnamagriculture.nongnghiep.vn/navetco-researches-to-produce-asf-vaccine-in-vietnam-d309586.html

[3] https://rescorp.org/scialog/mitigating-zoonotic-threats

throughout the United States. The FY2021 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 189 manuscripts in peer-reviewed journals.

Significant technology transfers were achieved with:

- 7 new inventions disclosures;
- The submission of 11 patent applications,
- 32 licenses issued for ARS patents;
- The establishment of 13 research agreements; and
- 6 new patents awarded.

The NP103 program also trained 70 students and post-doctoral candidates during FY2021.

New additions to the NP 103 team in 2021 are:

Dr. Bailey Arruda, Research Veterinary Medical Officer, joined the Virus and Prion Research Unit, Ames, Iowa.

Dr. Abhijeet Bakre, Research Microbiologist, joined the Exotic and Emerging Avian Viral Disease Research Unit, Athens, Georgia.

Dr. Steven Conrad, Biologist, joined the Endemic Poultry Viral Diseases Research Unit, Athens, Georgia.

Dr. Damarius Fleming, Zoologist, joined the Animal Parasitic Diseases Laboratory, Beltsville, Maryland.

Dr. Chang-Won Lee, Microbiologist, joined the Exotic and Emerging Avian Viral Disease Research Unit, Athens, Georgia.

Dr. Sujit Mohanty, Veterinary Medical Officer, joined the Endemic Poultry Viral Diseases Research Unit, Athens, Georgia.

Dr. Lindsay Wright-Piel, Molecular Biologist, joined the Animal Disease Research Unit, Pullman, Washington.

The following scientists retired in 2021:

Dr. Kay Faaberg, Microbiologist, Ruminant Diseases and Immunology Research Unit, Ames, Iowa.

Dr. John Neill, Research Microbiologist, Ruminant Diseases and Immunology Research Unit, Ames, Iowa.

Dr. Karen Register, Research Microbiologist, Ruminant Diseases and Immunology Research Unit, Ames, Iowa

Dr. Timothy Reinhardt, Research Animal Scientist, Ruminant Diseases and Immunology Research Unit, Ames, Iowa.

Dr. Joseph Urban, Jr., Microbiologist, Animal Parasitic Diseases Laboratory, Beltsville, Maryland.

Dr. Dante Zarlenga, Research Microbiologist, Animal Parasitic Diseases Laboratory, Beltsville, Maryland.

The distinguished record of these scientists is recognized worldwide and they will be sincerely missed at NP 103.

The following scientists in NP 103 received prominent awards in 2021:

Dr. John Dunn received the American Association of Avian Pathologists P.P. Levine Award for 2020 as the senior author of the best paper published in the journal *Avian Diseases*.

Dr. Hyun Lillehoj was inducted into the Hall of Honor for the American Association of Avian Pathologists of the American Veterinary Medical Association in recognition of distinguished contribution to poultry health.

Dr. Mitchell Palmer was awarded the 2021 Federal Partnership Award by the United States Animal Health Association (USAHA). The USAHA Federal Partnership Award recognizes federal employees that have demonstrated commendable service to the betterment of animal health in the United States.

Dr. Erica Spackman received the Phibro Animal Health Excellence Award in Poultry Research from the American Association of Avian Pathologists. Dr. Spackman has helped create diagnostic tests for viral diseases of poultry, including a real-time reverse transcriptase–polymerase chain reaction assay for avian influenza. She has also created RT-PCR diagnostic tests for Newcastle disease, avian astrovirus, avian reoviruses, turkey coronaviruses, and avian rotavirus.

Dr. David Suarez received the ARS Southeast Area Scientist of the Year Award for his outstanding and sustained research on the Avian influenza virus (AIV), Newcastle disease virus (NDV) and emerging exotic diseases in poultry.

Dr. Amy Vincent was elected as a Member of the National Academy of Medicine for her groundbreaking research that led to improved vaccines and surveillance for swine influenza.

Research Results:

<u>COVID-19 Impacts:</u> 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: Foreign Animal Diseases

Analysis of United States vulnerabilities to introduction of Japanese encephalitis virus.
Foreign Arthropod Borne Animal Disease Research Unit
National Bio and Agro-defense Facility
Manhattan, Kansas

Japanese encephalitis virus (JEV) is a mosquito-transmitted virus that can infect multiple animal species and cause severe disease and death in humans. JEV is endemic in Asia and the Pacific region but is not currently present in the United States. However, JEV has demonstrated the ability to spread to new geographic regions, and previous studies determined the United States is at high risk for JEV introduction. ARS researchers in Manhattan, Kansas, collaborated with Kansas State University researchers to better understand the challenges and consequences associated with potential JEV introduction into the United States. Results highlighted several areas of vulnerability, including increases in wild animal populations that can transmit the disease, illegal animal importation and movement, the reduction in mosquito control in regions with highest risk, and changing patterns of viral strains. Identifying these gaps can help target animal and mosquito monitoring to detect an incursion of JEV more rapidly. This information will also be useful to direct future research, such as studies of the effectiveness of existing vaccines against different strains of JEV. Efforts such as these can reduce the potential consequences of a JEV incursion in the United States.

Problem Statement 1B: Emerging Diseases

A swine-origin H3N2 influenza virus closely related to human H3N2v demonstrated transmission from swine to ferrets.
Virus and Prion Research Unit, National Animal Disease Center
Ames, Iowa

The transmission of influenza A viruses (IAV) from swine to humans occurs sporadically and is often associated with U.S. agricultural fairs. IAVs from swine that are detected in humans are called "variant" to differentiate from human seasonal IAV. During the 2016-

2017 influenza season, 61 H3N2 variant (H3N2v) cases were reported. ARS scientists in Ames, Iowa, compared the genomes of human H3N2v viruses and swine H3N2 viruses collected at the same 2017 state fair in Ohio, where ferrets were also directly infected with the H3N2 virus. In the study, pigs were infected with the virus and placed in an enclosure close to caged ferrets, which were chosen to test the spread of H3N2 because IAV transmission and infection in ferrets serves as model for human IAV transmission and infection. Results demonstrated that the swine H3N2 replicated in both pigs and ferrets exposed to the respiratory aerosols of infected pigs, showing potential transmission from pigs to susceptible ferrets. These results are the first to show a transmission model from swine to ferrets without modification to the virus, and highlight the need to reduce swine IAV at animal exhibits. This study also demonstrates the importance of continued surveillance, research, and collaboration on swine and human

Risk factors determined in the spread of high pathogenicity avian influenza during the 2014-2015 U.S. Outbreak.

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

The incidence and economic impact of avian influenza in poultry remains high worldwide. From December 2014 through mid-June 2015 an H5 subtype highly pathogenic avian influenza (HPAI) virus, which originated in Asia and spread to North America by migratory birds, caused the worst animal disease outbreak in the history of the United States. Approximately 49.6 million commercial birds died or had to be euthanized. This eradication effort cost more than $1 billion and the impact to the U.S. economy was more than $3.2 billion. ARS researchers in Athens, Georgia, and University of Georgia and the University of Connecticut collaborators found that the HPAI outbreak among poultry farms in the midwestern United States was influenced by agricultural and geographic factors. After initial introduction of the HPAI virus into the poultry industry, no further introductions, such as from a wild bird reservoir or long-distance movement, were necessary to explain the continuation of the outbreak from March to June 2015. Additionally, evidence suggests that the proximity of farms increased the chances of viral movement between two locations. While many theories could explain the transmission of virus among poultry farms, road density was found to be an important factor of virus movement, and human-based transportation played a key role. This information is critical in understanding the epidemiology of HPAI viruses and developing methods for prevention and control of the disease.

Component 2: Antimicrobial Resistance

Problem Statement 2B: Alternatives to Antibiotics

Development of a single novel vaccine for two specific bovine respiratory disease pathogens.

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

Antibiotics are commonly used to treat respiratory disease in cattle. Currently, there is no commercially available vaccine against *Mycoplasma bovis*. A novel vaccine against two bovine disease pathogens *Mannheimia haemolytica* and *Mycoplasma bovis* was developed and tested by ARS scientists in Ames, Iowa. In calf vaccination/challenge studies it was demonstrated that a single intranasal dose of the vaccine confers protection against respiratory disease, and the associated symptoms of disease, following challenge with virulent *Mannheimia* as well as following challenge with virulent *Mycoplasma*. The protection was associated with major reductions in the levels of disease-causing organisms in lung tissue following challenge. The vaccine also conferred significant protection against *M. bovis*-induced polyarthritis and middle ear infection, major economically important signs of disease. The vaccine is being further developed as a potential commercial product. These findings will be used by veterinarians, scientists and vaccine manufacturers seeking to reduce bovine respiratory diseases.

Component 3:   Zoonotic Bacterial Diseases

Problem Statement 3B: Leptospires

Genetic manipulation of pathogenic leptospires.

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

Leptospira bacteria are zoonotic pathogens which cause reproductive losses and clinical diseases in numerous hosts. Performing targeted gene mutations to create attenuated strains is difficult in Leptospira. Using the CRISPR/Cas 9 system, ARS scientists in Ames, Iowa, created recombinant strains of Leptospira in which immunogenic outer membrane proteins were genetically modified. This technology will be important for future vaccine development. Complete silencing of LipL32, LigA, and LigB genes were achieved, revealing for the first time that Lig proteins are crucial for disease pathogenesis. This work will be of interest to stakeholders and researchers who want to use leptospirosis vaccine development as a faster, more economical approach for new vaccine candidate creation.

Component 4: Respiratory Diseases

Problem Statement 4A: Bovine

Bison and Simmental genomes released.

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

The first reference-quality genome assemblies for the iconic North American Yellowstone bison and the Simmental cattle breed have been made publicly available. Animal genome assemblies provide genetic “blueprints” for how an animal develops and passes on genetic information to their offspring. Researchers in Clay Center, Nebraska, used in vitro fertilization to cross a bison bull with a Simmental cow. The hybrid animal and its purebred parents were fully sequenced to produce a complete, highly accurate genome assembly for each species. The bison assembly will be used in conservation efforts to maintain genetic diversity and to study bison evolution by comparing DNA obtained from fossil specimens sampled from permafrost in the Arctic Circle. The Simmental cattle assembly will contribute to an international effort to survey existing cattle breeds around the world to preserve their genetic diversity and identify variation useful for improving beef and dairy traits in different environments. These genomic tools will help to speed genetic progress and avoid counterproductive breeding before it happens, providing another means for increasing beef and dairy production important for food security, while maximizing environmental and economic sustainability.

Emerging bovine viral diarrhea virus strains 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 in the BVDV1 species but was not of the BVDV1a or BVDV1b groups that were known to be in the United States. Characterization of this virus was important to maintain vigilance for emerging pestiviruses and to determine the effectiveness of current bovine vaccines in protecting against these viruses. ARS scientists at 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 antisera raised against BVDV strains that are found in commercial bovine vaccines. This showed that the antibodies in these antisera recognized this BVDV1i strain but at a reduced level. This research suggests that current vaccines can provide some protection against emerging BVDV1 but further studies are warranted to confirm this observation.

Problem Statement 4B: Porcine

Streptococcus equi subspecies zooepidemicus causes disease in healthy, conventionally raised pigs.
Virus and Prion Research Unit, National Animal Disease Center
Ames, Iowa

Streptococcus equi subspecies zooepidemicus (SEZ) was isolated from pigs that died during multiple high mortality events in 2019; from 30 to 50 percent of exposed pigs developed severe infections or died. Before 2019, SEZ was not considered an important cause of disease in pigs. As part of emergency response research, ARS researchers in Ames, Iowa, in collaboration with Iowa State University and the APHIS National Veterinary Services Laboratories determined SEZ causes disease in healthy, non-stressed sows and 4-month-old pigs. When scientists exposed pigs to a 2019 SEZ field isolate, the pigs developed severe disease. This research confirmed that healthy, conventionally raised pigs can develop SEZ disease without stress or coinfection, which showed that the general swine population is susceptible to SEZ infection. These findings are important in evaluating the risk of SEZ to the swine industry.

Component 5: Priority Production Diseases

Problem Statement 5A: Johne's Disease

New Johne's vaccine for cattle.
Infectious Bacterial Diseases Research Unit, National Animal Disease Center
Ames, Iowa

Johne's disease, a serious disease of dairy cattle, is caused by *Mycobacterium avium subsp paratuberculosis* (MAP). ARS researchers in Ames, Iowa, conducted trials in dairy calves to test a new sub-unit Johne's disease vaccine containing a cocktail of recombinant proteins. In two trials, the highest dosages of the vaccine significantly reduced (MAP) colonization of intestinal tissues and resulted in the greatest reduction in infection. The vaccine also reduced fecal shedding of the pathogen, which is important for stopping on-farm transmission. Data has been used to support the patent application for this vaccine. These results will be of interest to producers, regulatory personnel, and researchers interested in intervention strategies for preventing Johne's disease in domestic livestock.

Component 6: Parasitic Diseases

Problem Statement 6A: Enteroparasitic Diseases

Better dewormers for small ruminants.
Animal Parasitic Diseases Laboratory
Beltsville, Maryland

Anthelminthic drug resistance cripple attempts to deworm sheep and goats which can lead to increased morbidity and mortality. Veterinarians and farmers urgently need new tools to protect animal health and ensure the productivity of small ruminant farms. Therefore, ARS researchers in Beltsville, Maryland, worked with university collaborators to refine a new, safe, cost-effective method to produce a potent therapeutic paraprobiotic called Inactivated Bacterium with Cytosolic Crystals (IBACC). Whereas a probiotic contains live microbes, a paraprobiotic contains inactivated microbes to enhance health. When given to sheep, three doses reduced the number of eggs shed by 90 percent, the total number of worms by 72 percent, and the number of female worms by 96 percent. Biologists think worms will face difficulty evolving resistance to this therapy. Once commercialized, this approach has enormous potential to benefit livestock producers.

Component 7: Transmissible Spongiform Encephalopathies

Problem Statement 7C: Diagnostics, Detection, and Prevention

Differing genetic backgrounds of prion disease sources do not affect likelihood of disease progression.

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. 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 for detection and has been used to differentiate prion diseases through differences in the rate of misfolding. ARS scientists in Ames, Iowa, completed a study comparing the relative rates of misfolding in RT-QulC from different sources of chronic wasting disease using both human and bank vole prion protein as the substrate for amplification. Regardless of the chronic wasting disease source and genotype, the bank vole substrate was equivalently sensitive indicating the utility of this substrate for the detection of CWD regardless of host or genotype. Similarly, with human prion protein substrate no differences were found indicating that at the level of conversion of the human prion protein to the fibril form conformation all tested CWD isolates are equivalent. From a diagnostic perspective this further justifies the use of bank vole prion protein as a universal substrate and indicates that regardless of genotype of the CWD source the risk to humans is likely the same. Both results provide important information for knowledge based regulatory decisions by state and federal agencies.