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 goals of the research mission are to produce knowledge and technology to reduce economic losses from infectious, genetic, and metabolic diseases. Dr. Cyril G. Gay, National Program Leaders (NPL), Animal Health, manages the program.

The Animal Health National Program will initiate a new five-year national program cycle Fiscal Year (FY) 2017. The Animal Health National Program currently will include 38 core research projects, with the support of 102 scientists located at 9 research sites throughout the country. The ARS research budget for the Animal Health Program FY 2016 was $70 million. Twenty two new inventions were disclosed and 7 patents awarded. Additional technology transfer included 55 Material Transfer Agreements and 11 Material Transfer Research Agreements.

New additions to the NP 103 team in 2016 are:

Dr. Taejoong Kim, Athens, Georgia, joined the Avian Disease and Oncology Laboratory as a Research Veterinary Medical Officer.

Dr. Leela Noronha, Manhattan, Kansas, joined the Center for Grain and Animal Health Research as a Veterinary Medical Officer.

The following scientists retired from the ranks in NP 103:

Dr. Raymond Fetterer, Animal Parasitic Disease Laboratory, Beltsville, Maryland.

Dr. Laszlo Zsak, Endemic Poultry Viral Diseases Research Unit, Athens, Georgia.

The distinguished record of service of these scientists is recognized world-wide and they will be missed at NP103.

The following scientists in NP 103 received prominent awards in 2016:

Dr. Joan Lunney, Beltsville, Maryland, was promoted to Supergrade.
Dr. Leela Noronha, Manhattan, Kansas, received a Certificate of Commendation for Outstanding Support of Accomplishment of Missions of Defense Intelligence Agency.

Dr. David L. Suarez, Athens, Georgia, received the 2016 American Veterinary Medical Association Public Service Award.

Dr. David E. Swayne, Athens, Georgia, received the 2016 Inaugural Class of the American Association of Avian Pathologists Hall of Honor Award.

Dr. Dante Zarlenza, Beltsville, Maryland, was elected Vice President of the American Association of Veterinary Parasitology.

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

**Recoding classical swine fever virus (CSFV) structural glycoprotein E2 and its potential as a vaccine candidate**
A DNA codon is a series of three nucleotides that codes for specific amino acids, which are the building blocks that comprise proteins. Some amino acids have more than one codon, resulting in what is called synonymous codon usage. Relative synonymous codon usage (RSCU) refers to the phenomenon during which some synonymous codons are used more often than others. ARS scientists at the Plum Island Animal Disease Center, Orient Point, New York, determined that the E2 glycoprotein, a determinant of virulence of the classical swine fever virus (CSFV), had a similar codon usage as pigs (Sus Scrofa), its natural host. They explored the effect of just switching native codons in E2 for the less frequently used codons. Their studies showed that changing to synonymous codons in E2 resulted in the full attenuation of CSFV in pigs, and demonstrating that the genetically-altered CSFV no longer caused disease. This phenomenon is thought to occur because changing the codon impacts the ability of the gene to be expressed. Interestingly, when ARS scientists explored the potential use of this phenomenon to generate a vaccine strain, they found that the altered virus was able to protect animals against the disease. By using synonymous codons and not changing a single amino acid, this potential vaccine leaves all natural antigenic epitopes intact. The benefits of producing vaccines using this technology is that the antigenic profile of the virus remains intact, which is important for inducing a protective immune response. Additionally by changing the nucleotide composition, genetic markers are now available that could be used to differentiate between vaccinated and infected animals.

**Mechanisms and pathogenesis of the Foot-And-Mouth Disease carrier state in cattle**
Foot-and-Mouth Disease Virus (FMDV) is the most infectious disease of animals and impacts food security in much of the world. A key challenge in the control of FMDV is that 50 percent of vaccinated cattle become persistently infected. Persistent FMDV infection in cattle is largely responsible for the massive depopulation of animals because of the fear that these animals, although asymptomatic with no clinical signs, may spread
FMDV and infect other animals. There is currently very little knowledge about the mechanisms that lead to this carrier stage. ARS scientists at the Plum Island Animal Disease Center, Orient Point, New York, reported in the Journal of Virology new aspects of viral persistence and host mechanisms associated with chronic asymptomatic FMDV infection. One key finding was that the divergence between carrier animals and FMDV-free animals occurs as early as 10 days post infection. Microscopic localization of the virus indicated persistent infection of specific regions of the nasopharyngeal mucosa. These findings provide new insights into paths that may be taken to develop vaccines that could prevent FMDV carrier animals. Having a vaccine to prevent FMD-persistent infections would support the implementation of a "vaccine-to-live" strategy and eliminate the needless slaughter of vaccinated animals.

The potential role of prion genetics in cattle with classical versus atypical Bovine Spongiform Encephalopathy

In 2006, a case of atypical bovine spongiform encephalopathy (BSE-H) was diagnosed in a cow that was associated with a heritable genetic mutation in the bovine prion protein gene (PRNP). Unlike classical BSE, which is caused by feeding cattle with contaminated BSE material, it is thought that atypical BSE cases may occur spontaneously in cattle due to genetic mutations in the PRNP. ARS scientists at the National Animal Disease Center in Ames, Iowa, conducted a series of pathogenicity studies and showed that the survival time of the cattle with the genetic mutation inoculated with BSE-H was shorter (10 months) than cattle without the mutation (18 months). This genetic effect was not observed when cattle, with or without the genetic mutation, were inoculated with classical BSE. Their survival time was 26 months, regardless of whether the cattle had the genetic mutation or not. The results of these studies demonstrate that the genetic mutation associated with atypical BSE exhibits a number of features that differ from classical BSE. Understanding the association between this genetic mutation and BSE provides important information on the potential public health risk of atypical BSE.

Differences in T-Lymphocytes and cecal microbiome community during Marek’s disease virus infection are associated with genetic resistance to Marek’s disease

Marek’s disease (MD) is an important oncogenic disease of chickens caused by Marek’s disease virus (MDV). In previous studies, ARS scientists demonstrated that certain genetic lines are resistant to MD. Recent interest in the role of the microbiome in preventing diseases led ARS scientists at the Avian Diseases and Oncology Laboratory, East Lansing, Michigan, in collaboration with Michigan State University scientists, to investigate the potential role of the microbiome in MD resistance. The results of this research showed differences in two chicken genetic lines, one resistant and the other susceptible to MD. Splenic helper CD4+ T cells were similar; however, cytotoxic CD8 sub-populations were different with a greater number of lymphocytes with cell surface markers associated with innate immunity in the MD resistant chicken line. Interestingly, the microbiome composition was different between resistant and susceptible birds. With MDV challenge, both chicken lines showed lower numbers of beneficial Faecalibacterium species and increased number of Lactobacillus species. Metabolic profiles between chicken types were similar but with MDV challenge, there were differences in metabolism in both chicken lines, with amino acid metabolism impacted in
resistant birds and lipid metabolism in susceptible birds. These results provide insights into differences in the immune response of MD-resistant chickens and potential interplay with the microbiome during infection with an oncogenic virus. Information on the role of the microbiome in preventing diseases may be used in the future to reduce the loss from Marek's disease.

**Presence of Vaccine-Derived Newcastle Disease Viruses in Wild Birds**

Of all the factors influencing disease emergence, likely the most substantial is the loss of ecological species barriers, permitting opportunistic pathogens access to wildlife. ARS scientists at the U.S. National Poultry Research Center, Athens, Georgia, in collaboration with University of Georgia scientists demonstrated the repeated isolation of vaccine-derived Newcastle disease viruses from different species of wild birds across four continents from 1997 through 2014. The data indicate that at least 17 species from 10 avian orders occupying different habitats excrete vaccine-derived Newcastle disease viruses. Examining the extent of vaccine derived Newcastle disease virus from live vaccines, including recently developed recombinant vaccines, transferred from poultry into wild birds is crucial because the downstream epidemiological consequences of such spillovers are still unknown. Circulating live vaccine viruses present additional risks such as reversion of virulence and recombination with wild-type strains. In addition, the immune response of wild birds induced by infection with vaccine strains may provide selective pressures resulting in viral antigenic drift or increased virulence. The finding of live attenuated Newcastle disease virus vaccines in other avian species provides important evidence that the use of these vaccines should be monitored to assess their potential impact on the environment and the emergence of new viral strains.

**H9N2 low pathogenic avian influenza viruses with zoonotic potential in Pakistan**

Significant economic losses from bird mortality and decreased egg production have resulted from H9N2 low pathogenic avian influenza virus (LPAIV) infections in poultry across North Africa, the Middle East and Asia. This group of viruses has also caused sporadic infections in mammalian species, including human beings, and has been associated with some specific genetic changes that suggests increasing pandemic potential. The H9N2 LPAIVs have been endemic in Pakistani poultry since 1996, but no new viruses have been reported since 2010. Because novel genotypes of Pakistani H9N2 contain mammalian host-specific markers, recent surveillance is essential to better understand any continuing public health risk. ARS scientists at the U.S. National Poultry Research Center, Athens, Georgia, in collaboration with Pakistani scientists reported this year the characterization of four new H9N2 LPAIVs, three from 2015 and one from 2012. All of the viruses tested in this study belonged to viruses originating in the Middle East. Importantly, these viruses all contained mammalian host-specific markers, suggesting that Pakistan avian H9N2 viruses have the capacity to infect mammals. Continued active surveillance in poultry and mammals is needed to monitor the spread and understand the potential for zoonotic infections by these H9N2 LPAIVs.

**Automated vaccine delivery system to improve biosecurity in poultry houses**

Traditional methods of vaccinating poultry often involve an operator entering the poultry house to spray vaccine using a backpack-mounted device, which increases the likelihood
of pathogens being inadvertently brought into the barn with equipment and increasing biosecurity risks. Although vaccines are often added to poultry water supplies, there can also be problems with contamination using this method of vaccination. To solve these problems, ARS scientists at the Poultry Research Unit, Mississippi State, Mississippi, developed a new, fully automated system for spraying the vaccines inside the barns. The idea behind the machine is to trigger birds to drink from the water lines using changes in lighting. After the birds go to the water lines, vaccine spray nozzles are lowered from the ceiling and spray the birds. The nozzles are strategically placed above the water lines, to ensure maximum coverage over the birds. The target is the important eye area, where scientists know that vaccines are easily absorbed by the birds, but the vaccine will also be picked up by the birds through preening and contact with other birds. The scientists compared the performance of the automated system against a three-man vaccination crew with backpack sprayers using a combination infectious bronchitis and Newcastle disease vaccine. The results of the blood samples taken from automatically vaccinated flocks showed improved immune protection against the viruses compared with the backpack method, as well as reducing biosecurity risks and the number of personnel required to vaccinate a flock.

**Sensitive and specific detection of classical scrapie prions in the brains of goats**

Scrapie is a transmissible spongiform encephalopathy that causes fatal neurodegenerative disorders in goats and sheep. “Real-time quaking-induced conversion” is a rapid, specific and highly sensitive detection assay used to detect low levels of abnormal scrapie prion proteins (PrPSc). Although this sensitive assay has been successfully used to detect abnormal prion proteins in various tissues from humans and animals, including sheep, tissues from goats infected with scrapie have not yet been tested. ARS scientists at the Animal Diseases Research Unit, Pullman, Washington, in collaboration with scientists at Washington State University, evaluated whether abnormal prion proteins could be detected in the brain tissues of goats with scrapie using this sensitive assay, optimized reaction conditions to improve scrapie detection in goats, and compared the performance of this sensitive assay for the detection of scrapie with the more commonly used prion detection methods. ARS scientists further optimized assay conditions for sensitive and specific detection of goat scrapie in clinical animals. The results from these studies provided good discrimination between scrapie-infected and normal goat brain samples. Importantly, these studies indicated that this highly sensitive assay was at least 10,000-fold more sensitive than the commonly used prion detection methods for the detection of scrapie activity in goat brain samples.

**Using genomics to identify novel antimicrobials**

There is a critical need in animal agriculture to develop novel antimicrobials and alternative strategies that will help to reduce the use of antibiotics and address the challenges of antimicrobial resistance. High-throughput gene expression analysis is providing new tools that are enabling the discovery of host-derived antimicrobial peptides. One example are the NK-lysins that were first described in mammals and are also found in avian species, which have been shown to have antimicrobial activities that could potentially be used to control important poultry pathogens. ARS scientists at the Animal Bioscience and Biotechnology Laboratory, Beltsville, Maryland, demonstrated
alterations following chicken NK-lysin binding to coccidia and Escherichia coli membranes, indicating damage and disruption of cell membranes, suggesting that NK-lysin kills pathogenic protozoans and bacteria by direct interaction. DNA analysis revealed that chicken NK-lysin peptides derived from certain genes were more effective at killing pathogens than those derived from other genes, which could potentially result in certain genetic lines of poultry being more resistant to diseases. Although these host-derived antimicrobial peptides may not, by themselves, be able to replace the antibiotics currently used in animal production, their use as specific treatments based on their known mechanisms of action is showing promising results.

**Senecavirus A is one of the causative agents of swine vesicular disease**

Idiopathic vesicular disease in swine is a diagnosis made when none of the pathogens known to cause swine vesicular disease [vesicular exanthema virus, swine vesicular disease virus, vesicular stomatitis virus, and foot-and-mouth disease virus (FMDV)] have been detected in a clinical case. Occasionally, an emerging virus called Senecavirus A (SVA) has been detected in cases of idiopathic vesicular disease, raising the possibility that SVA infection could cause vesicular disease in swine. This assumption was strengthened by the recent emergence of idiopathic vesicular disease in Brazil and the United States from which there was frequent detection of SVA. For the first time, ARS scientists at the National Animal Disease Center, Ames, Iowa, in collaboration with scientists at Iowa State University have been able to experimentally induce clinical signs and gross lesions in nursery age pigs inoculated with SVA, demonstrating a causative relationship between SVA infection and vesicular disease in susceptible pigs. This is noteworthy because SVA disease is clinically indistinguishable from other vesicular diseases of swine, especially FMD, a highly transmissible livestock disease that can cause devastating economic losses to farmers. Developing animal disease models is key to animal health research to understand pathogenicity and disease transmission and test experimental vaccines that could be used to prevent the disease.