Animal Health (NP 103) Annual Report for 2017

Introduction

Vision: The vision for Agriculture Research Service (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 Leader, Animal Health, manages the program. As of November 2017 (FY2018), Dr. Roxann B. Motroni joined the Office of National Programs as the co-National Program Leader for animal health and will also manage and lead the research 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 94 scientists located at 9 research sites throughout the country. The ARS research budget for the Animal Health Program FY 2017 was $65 million. Scientists working in the program published 208 manuscripts in peer-reviewed journals. A total of 12 new inventions were disclosed and 6 patents awarded. Additional technology transfer included 79 Material Transfer Agreements and 8 Material Transfer Research Agreements.

New additions to the NP 103 team in 2017 are:

Dr. Eugenio Abente, Ames, Iowa, joined the Virus and Prion Research Unit as a Research Microbiologist.

The following scientists in NP 103 received prominent awards in 2017:

Dr. Jitender Dubey, Beltsville, Maryland, was honored as a finalist for the Samuel J. Heyman Service to America Medal.

Dr. Don Knowles, Pullman, Washington, received the USDA-ARS Research Leadership and Center Directorship Award.

Dr. Joan Lunney, Beltsville, Maryland, was named an International Society of Animal Genetics (ISAG) Fellow.

Dr. David Suarez, Athens, Georgia, received the Excellence in Technology Transfer Project Award "Recombinant Highly Pathogenic Avian Influenza Virus Vaccine" South East Regional Award from the Federal Laboratory Consortium.

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

A computational tool to characterize the antigenic diversity of swine influenza viruses
Infection with swine influenza A viruses (IAV) is one of the most important respiratory diseases of swine and is the second most common viral diagnosis of respiratory disease in swine in the United States. Furthermore, the global diversity of swine IAV creates substantial risks for both human and swine populations and could be a major contributor to future outbreaks and potential pandemics in humans. There is therefore a keen interest in improving the control of IAV in swine through vaccination. A significant barrier to improve the efficacy of vaccines is lacking the computational expertise to analyze and characterize the hemagglutinin (HA) gene of IAV to properly match vaccines to field strains. The HA protein is a major component of vaccines and target to induce a protective immune response. ARS scientists working at the National Animal Disease Center in Ames, Iowa, in collaboration with the global network of animal influenza virus experts (OFFLU), have developed a computational tool that automatically classifies HA gene sequences from swine IAV isolates. This open-access tool is now widely available and will aid swine producers, veterinarians, vaccine manufacturers, and IAV vaccine researchers in selecting vaccine strains to match the strains that are currently circulating on swine farms.

Early warning strategies for vector-borne animal disease outbreaks
Vesicular stomatitis virus (VSV) is a vector-borne animal pathogen that causes lesions and other symptoms that are clinically indistinguishable from Foot-and-Mouth Disease (FMD), and is one of the most common vesicular diseases affecting livestock (domestic horses, cattle, pigs) throughout the Americas. VSV is also zoonotic and can cause mild flu symptoms in people. Despite numerous epidemiological studies, there is currently limited understanding of the factors responsible for the VSV outbreaks that have occurred in the United States every decade since 1916. VSV is an excellent research model for understanding and potentially predicting vector-borne animal disease outbreaks because it is complex and there is a lack of understanding of ecological patterns, or the role of environmental factors that may contribute to disease outbreaks through time at local and regional scales. Therefore, scientists with diverse scientific expertise from five locations across the United States (Plum Island Animal Disease Center, Orient Point, New York; Arthropod-Animal Disease Center, Manhattan, Kansas; Jornada Experimental Range Unit, Las Cruces, New Mexico; Rangeland Resources and Systems Research Unit, Cheyenne, Wyoming; Center for Epidemiology and Animal Health, Fort Collins, Colorado), collaborated on developing early warning strategies for VSV. Coupling big data-model integration with

human and machine learning, ARS scientists evaluated the relative importance of a large and diverse suite of environmental, insect and livestock variables to patterns in VSV disease outbreaks. Given information on latitude, elevation, and long-term precipitation, veterinarians and livestock owners should now be able to monitor their local conditions to determine the likelihood that VSV will occur in each month of the year. These early VSV warning strategies could also inform public health preparedness and response to other emerging zoonotic vector-borne diseases.

Protecting livestock producers from the threat of foot-and-mouth disease (FMD)
Foot-and-mouth disease (FMD), a highly contagious disease affecting cloven-hoofed animals such as cattle, pigs and small ruminants is considered a major global threat to animal agriculture. Although FMD was eradicated from the United States in 1929, it has been estimated that a reintroduction would result in $200 billion in lost revenue over 10 years. While FMD diagnostics and vaccines have been used effectively in controlling the disease, significant gaps remain in the availability of effective veterinary medical countermeasures suited for use in the United States. Therefore, ARS scientists working at the Plum Island Animal Disease Center (PIADC) have dedicated resources and made significant breakthroughs in developing new improved veterinary countermeasures to detect, prevent, and control FMD should an incursion ever occur in the United States. The first is an attenuated vaccine platform called the “leaderless” FMDLL3B3D vaccine. This vaccine mimics the immune response of commercially available inactivated FMD vaccines, which are made with virulent wild type virus strains. But unlike commercial FMD vaccines, the FMDLL3B3D vaccine virus strains are fully attenuated; thus enabling their safe production in the United States. The importance of the vaccine is that it's beneficial immune capability is created without the risk of potentially causing a devastating FMD outbreak should the vaccine virus escape from a manufacturing facility. Furthermore, the FMDLL3B3D vaccine virus platform was genetically-engineered with two negative markers to allow the differentiation of infected from vaccinated animals (DIVA). Such differentiation is paramount in the recovery phase of a disease outbreak to establish disease free status, and obtain approval to resume the export of agricultural products. This FMD vaccine is now in the advanced development phase with a commercial partner, with approval from regulatory authorities for manufacturing and distribution expected within two years. The second breakthrough is a novel DIVA companion diagnostic test for the FMDLL3B3D vaccine that was developed through a consortium of academic, industry, and federal agencies comprised of APHIS, ARS, and DHS scientists working at the PIADC. This is the first licensed FMD diagnostic kit approved for manufacturing on the U.S. mainland. Together, the FMDLL3B3D vaccine and companion diagnostic test kit increase national security by providing animal health first responders with important new tools to mitigate the potentially catastrophic economic impact of an FMD outbreak.

Transmission of foot-and-mouth disease virus (FMDV) from persistently infected cattle
Scientists at the ARS Plum Island Animal Disease Center continue to decipher the biological mechanisms that results in foot-and-mouth disease virus (FMDV) persistently infected cattle. Over half of FMDV infected cattle, whether vaccinated or not, become persistently infected carrier animals. This phenomenon is established when FMDV is

maintained in their tissues for 28 days or more after infection. Carrier animals eventually clear the infection; however, the length of time required to clear the infection varies greatly. Although a large proportion of infected cattle become carriers, it is unclear whether carrier cattle can spread the infection to naïve cattle. As a result, FMD-free countries experiencing an FMD outbreak destroy carrier animals due largely to the perceived risk of transmission from carrier animals. However, destroying carrier animals is not economically feasible in the large majority of FDM-endemic countries. Therefore, ARS scientists investigated the potential for transmission of FMDV from persistently infected cattle to naive cattle under typical husbandry conditions. Results from a research collaboration with FMD scientists in Vietnam showed that no transmission occurred during six months of contact between FMD carrier and naïve cattle. These results will inform FMD response policy in the event of a large-scale outbreak. The average duration of the carrier state in this study was 27.7 months. The results of this study suggests that the duration of persistent infection in cattle may be longer than previously recognized, but the risk of transmission is low. Additionally, ARS evaluated one carrier animal for a 12 months period and fully sequenced the genome of seven viruses recovered from that animal during the study period. The analysis of the genome sequence of these seven viruses showed that a number of mutations occurred during the carrier stage. This is the first report of complete sequences of FMDV isolated from one persistently infected animal under natural conditions. The characterization of these viruses provides insights into within-host evolution of FMDV during persistent infection and has implications for FMD control in areas that are endemic for the disease.

New vaccine to fight deadly African disease in pigs

African Swine Fever Virus (ASFV) is a deadly disease effecting swine, causing near 100% mortality, trade restrictions and significant economic losses globally. As its name indicates, the disease occurs in Africa. However, in 2007 the disease appeared in the Caucasus (Republic of Georgia), and has subsequently spread into Russia and the Ukraine. ASFV continues to spread and is now present in multiple countries of Eastern Europe, including Poland and most recently the Czech Republic, posing an imminent threat to the European and global swine industries. Currently there are no vaccines to protect swine against ASFV. Therefore, ARS scientists at the Plum Island Animal Disease Center in Orient, New York, have developed an improved live vaccine that is safe and can protect swine as early as 2 weeks post vaccination. This is the first experimental vaccine shown to induce early protection against ASFV in swine. This vaccine could be used globally to protect swine from this deadly disease and also safeguard the United States pork industry against the increasing incursions of this devastating disease.

Component 2: Antimicrobial Resistance

Swine to human transmission of methicillin-resistant Staphylococcus aureus (MRSA)

Staphylococcus aureus is a common and sometimes devastating human pathogen that has the ability to acquire resistance to antibiotics resulting in methicillin-resistant *Staphylococcus aureus* (MRSA). Swine can carry strains of MRSA that do not appear to

cause disease in swine, but it is unclear whether livestock-associated (LA)-MRSA are a risk for humans. Therefore, ARS scientists at the National Animal Disease Center, Ames, Iowa, investigated the genetic mechanisms of antimicrobial resistance among swine LA-MRSA and human clinical MRSA isolates and found that swine isolates exhibited resistance to fewer antibiotics than MRSA isolates from humans with no swine contact. Furthermore, distinct genomic antimicrobial resistance elements were harbored by each group, with little overlap in shared antimicrobial resistance genes between swine LA-MRSA and human clinical MRSA isolates. These results suggest there are distinct populations of MRSA in swine and humans, antibiotic resistance is more prevalent in human strains, swine to human transmission is infrequent, and that LA-MRSA may not be a common zoonotic threat.

Effect of zinc in the selection of methicillin-resistant Staphylococcus aureus (MRSA)
Feeding zinc to young pigs to prevent diarrhea is a common practice in the swine industry. But public health concerns over this practice have increased because using zinc supplements has been partly attributed to the occurrence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) strains in Europe. Therefore, ARS scientists at the National Animal Disease Center, Ames, Iowa, along with collaborators at Iowa State University and University of Minnesota, compared the prevalence of zinc resistance genes in U.S. swine LA-MRSA isolates with their prevalence in MRSA isolates from humans with no swine contact. None of the swine MRSA isolates were resistant to zinc. In contrast, 21.9 percent of isolates from humans with no swine contact carried the zinc resistance gene, and 24.7 percent were resistant to zinc. These results suggest that the application of zinc in feed, has not led to an increase in zinc resistant MRSA isolates in swine populations, and that these LA MRSA are not the source of zinc resistant MRSA in humans.

Direct-fed microbials as an antibiotic alternative
With an increase in concerns regarding the development of antibiotic resistance and efforts to promote the judicious use of antibiotics in food-producing animals, there is a timely need for the development of viable alternatives to ensure and maintain optimal animal health and performance. Direct-fed microbials (DFMs), often referred to as probiotics, are a potential non-antibiotic replacement that has been studied extensively and used in commercial applications. DFMs are beneficial bacteria and often used as a feed supplement to promote gut health. Therefore, to better understand the health benefits of probiotics in enhancing gut health in poultry, and the mechanisms used by the non-pathogenic probiotic bacteria *Bacillus subtilis*, ARS scientists at the Animal Biosciences and Biotechnology Laboratory in Beltsville, Maryland, carried out extensive animal studies to show that certain Bacillus strains stimulate host innate immune responses, decrease harmful inflammatory responses, and promote gut integrity when used as a feed additive in young chickens. These results provide scientific evidence for the beneficial effects of probiotic bacteria and the potential use of some strains of *Bacillus subtilis* as a feed additive to promote gut health in commercial poultry production, and reduce the use of medically important antibiotics.

Component 3: Zoonotic Bacterial Diseases

Long duration antibiotic not effective against brucellosis in goats.
Whole herd treatment of infected goats with a long lasting antibiotic could be an effective treatment for reducing infection, clinical effects and disease transmission to humans. Therefore, ARS scientists at the National Animal Health Disease Center in Ames, Iowa, treated goats with a commercial antibiotic after experimental infection with *Brucella melitensis* and found that infection and abortion rates were not decreased. Although this antibiotic is expected to remain efficacious for 21 days after treatment, this study indicates that it would not provide economic or epidemiologic benefits under field conditions. This work eliminates a possible therapeutic approach for managing brucellosis in areas of high disease prevalence but it clearly shows that the use of antibiotics as an intervention strategy for brucellosis in goats is not effective.

Component 6: Parasitic Diseases

A new research tool for tick-transmitted diseases that eliminates the need for animals
Laboratory methods to enhance large-scale preparations of purified pathogens from infected tick vectors are paramount to advance research of tick-transmitted diseases such as bovine anaplasmosis, bovine babesiosis, Lyme disease, and Heartwater. But these methods require the use of live animals to propagate these pathogens. Therefore, scientists at the ARS Animal Diseases Research Unit in Pullman, Washington, designed and developed a novel continuous flow laboratory tick feeding system that facilitates isolating pure, free, and infectious tick-borne pathogens, thus eliminating the traditional method of using animals for isolating pure cultures of pathogens. Ticks are fed on a silicone membrane covering blood circulated at a constant temperature, mimicking living animals. The laboratory tick feeding system will be a useful method to improve live vaccine development for tick-borne diseases, to study pathogen-tick vector interaction, and the tick-mammalian host interface. This new laboratory method not only improves pathogen isolation but is responsive to the goal of improving animal welfare by replacement, reduction and refinement of animal use in the study of tick transmitted diseases.

Improving the accuracy of diagnostic tests for Bovine babesiosis
Bovine babesiosis (also known as cattle tick fever) is a tick-transmitted disease caused by the protozoan parasites *Babesia bovis* and *B. bigemina. Babesia* parasites can be transmitted by tick vectors to cattle of any age, which can result in 90% mortality in naive adults. Babesiosis was a significant problem in the southern United States until the eradication of the tick vector in the 1940's. The United States imports a million head of cattle yearly from Mexico, where Babesiosis and cattle fever ticks are present. The control measure used to prevent babesiosis from coming to the United States is treating all cattle arriving from Mexico with acaricides to eliminate cattle fever ticks. But, the recent discovery of acaricide resistant tick populations capable of transmitting *babesia species*, and the re-emergence and spread of cattle fever ticks by wildlife on the Texas - Mexico border is increasing the risk of the return of bovine babesiosis, which is a major

concern for the U.S. livestock industry. Therefore, scientists at the ARS Animal Diseases Research Unit in Pullman, Washington, have developed a modified diagnostic test with greater accuracy than the current standard tests to determine the infection prevalence of bovine babesiosis on the United States-Mexico border. This improved diagnostic test was fully developed in collaboration with a commercial partner and is now available for use to determine whether bovine babesiosis infections of cattle and wildlife are moving beyond the United States-Mexico border.

Novel screening system developed to identify anti-parasite molecules

A microfluidic device ("chip") was developed to record the rhythmic contraction of the pharynx of parasitic worms that control worm feeding. These electropharyngeograms (EPGs) can now be used to record responses from multiple worms per chip and can be used to evaluate novel drugs that target worm feeding to damage worm development. Therefore, ARS scientists at the Animal Parasitic Diseases Laboratory in Beltsville, Maryland, and colleagues at the University of California - San Francisco, George Washington University, and the University of Oregon validated this microfluidic technology using larval stages of parasitic human hookworms and the pig large round worm Ascaris suum against known anti-parasite drugs. Novel drugs are now being tested with this device. This showed that the microfluidic EPG platform provides a new tool for screening candidate drugs that can be further tested to eliminate parasitic worms from animals and humans.

Component 7: Transmissible Spongiform Encephalopathies

Swine are potential hosts for the scrapie agent

A naturally occurring prion disease has not been recognized in swine, but the agent of bovine spongiform encephalopathy does transmit to swine by experimental routes. Swine are thought to have a robust species barrier when exposed to the naturally occurring prion diseases of other species, but the susceptibility of swine to the agent of sheep scrapie has not been thoroughly tested. Therefore, ARS scientists at the National Animal Disease Center in Ames, Iowa, conducted this experiment to test the susceptibility of swine to U.S. scrapie isolates by intracranial and oral inoculation. Necropsies were done on a subset of animals at approximately 6 months post inoculation (PI): the time the pigs were expected to reach market weight. Remaining pigs were maintained and monitored for clinical signs of transmissible spongiform encephalopathies (TSE) until study termination at 80 months PI or when removed due to disease. Brain samples were examined by multiple diagnostic approaches, and for a subset of pigs in each inoculation group, bioassay in mice expressing porcine prion protein. At 6 months PI, no evidence of scrapie infection was noted by any diagnostic method. However, at 51 months of incubation or greater, five animals were positive by one or more diagnostic methods. Furthermore, positive bioassay results were obtained from all inoculated groups (oral and intracranial; market weight and end of study) suggesting that swine are potential hosts for the agent of scrapie.

Research Impact beyond Animal Health: Biomedical Research

The silver bullet: utilizing vesicular stomatitis virus to treat cancer

Vesicular stomatitis virus (VSV) is an animal pathogen that causes vesicular disease in horses, cattle and pigs. VSV is a bullet-shaped enveloped virus that grows rapidly producing powerful immune response that can be used as a vaccine to prevent and/or treat infectious disease and cancer in humans and animals. Modified VSV has been shown to replicate selectively in and kill cancer cells and were not pathogenic during clinical trials in humans and dogs. However, there is concern that these VSV vectors could be pathogenic and transmissible to farm animals (e.g. pigs). ARS scientists at the Plum Island Animal Disease Center, Orient, New York, collaborated with scientists at the Mayo Clinic, Rochester, Minnesota, and showed that VSV is safe not only to humans and dogs, but also in pigs. These studies pave the way for further development of this promising cancer therapy.