Animal Health (NP 103) Annual Report for 2018

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. Drs. Cyril Gerard Gay and Roxann Motroni lead the Animal Health National Program.

The Animal Health National Program currently includes 38 core research projects, with the support of 89 scientists located at 9 research sites throughout the country. The ARS research budget for the Animal Health Program FY 2018 was $75 million. Scientists working in the program published 197 manuscripts in peer-reviewed journals. A total of 16 new inventions were disclosed, 16 patents filed, and 9 new patents awarded. Additional technology transfer included 74 Material Transfer Agreements and two new Material Transfer Research Agreements.

New additions to the NP 103 team in 2018 are:

Dr. Steve Behan, Support Veterinary Medical Officer, joined the Arthropod-Borne Animal Diseases Research Unit, Manhattan, Kansas.

Dr. Dana Mitzel, Research Microbiologist, joined the Arthropod-Borne Animal Diseases Research Unit, Manhattan, Kansas.

The following scientists retired from the ranks in NP 103:

Dr. Eric Hoberg, Animal Parasitic Diseases Laboratory, Beltsville, Maryland.

Dr. Ray Waters, Infectious Bacterial Diseases Research, Ames, Iowa.

The distinguished record of these scientists is recognized world-wide and they will be missed at NP 103.

The following scientists in NP 103 received prominent awards in 2018:

Dr. Jonathan Artz, Orient Point, New York, received the award for "Best Talk Overall" for his presentation at the European Commission for the Control of Foot-and-Mouth

Disease (UFMD): Increasing Global Security in the Supply of Effective FMD Vaccines meeting in Puglia, Italy, on October 29-31, 2018.

Dr. Manuel V. Borca, Orient Point, New York, received the 2018 Dr. Daniel E. Salmon Award for Exemplary Achievement in Federal Veterinary Medicine at the 2018 USDA Awards Ceremony from the National Association of Federal Veterinarians.

Dr. Julia Ridpath, Ames, Iowa, received the American Association of Veterinary Laboratory Diagnosticians (AAVLD) Pioneer in Virology award for her achievements and contributions in the field of virology, particularly in diagnostic virology at the 61st Annual AAVLD meeting in Kansas City, Missouri.

Dr. David Suarez, Athens, Georgia, received entry to the American Association of Avian Pathologists Hall of Honor which recognizes AAAP members who have demonstrated significant contributions to the advancement of poultry health and contributions to the AAAP. He entered the Hall of Honor on July 16th, 2018.

Dr. Erica Spackman, Athens, Georgia, received the Bruce W. Calnek Achievement Award from the American Association of Avian Pathologists for her outstanding research contributions resulting directly or indirectly in a measurable, practical impact on the control of one or more important diseases of poultry, specifically the development of the real-time RT-PCR tests for avian influenza, improved collection methods for optimal sensitivity of pathogens, and improved methods for cost effective cleanup of premises with influenza infected birds. She received this award July 16, 2018.

Dr. Erica Spackman, Athens, Georgia, received the Golden Egg Award for service as the Chair of the Avian Influenza Subcommittee, Chair of the Technical Advisory Committee of the National Poultry Improvement Plan and "for encouraging the production of valuable, scientific and unbiased input by creating the optimum environment for healthy discussion." She received this award June 25, 2018.

Dr. David E. Swayne, Athens, Georgia, was recognized at the 10th International Symposium on Avian Influenza for outstanding veterinary science contributions to knowledge on avian influenza for prevention and control, whilst providing advice and leadership to government institutions and animal health organizations, Brighton, United Kingdom, on April 17, 2018.

Dr. David E. Swayne, Athens, Georgia, received 2018 XIIth International Veterinary Congress Prize, from the American Veterinarian Medical Association (AVMA) in Denver, Colorado, on July 13, 2018, for recognition of outstanding service by a member of the AVMA who has contributed to international understanding of veterinary medicine.

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

Transmission of Foot-and-Mouth Disease from Persistently Infected Carrier Cattle
Plum Island Animal Disease Center: Foreign Animal Disease Research Unit
Orient Point, New York
Control and eradication of foot-and-mouth disease virus (FMDV) are impeded by the existence of a persistent, subclinical phase of infection in ruminants; animals with this status are referred to as carriers. Although the epidemiological significance of these FMDV carriers is uncertain, fifty percent of cattle infected with FMDV become carriers, resulting in a substantial impact on the international trade of animal products. ARS scientists at the Plum Island Animal Disease Center investigated whether oral fluids from these carrier animals could transmit FMDV to cattle and pigs. Results from these studies showed that naïve cattle inoculated orally with fluids harvested from carrier animals developed clinical FMDV. In contrast, pigs exposed by inoculation of the same fluid samples harvested from the same persistently infected carrier cattle did not develop FMDV. These findings indicate that there is demonstrable contagion for cattle associated with FMDV carrier cattle. The results from this investigation provide new information that should improve response plans for FMDV control and eradication.

Susceptibility of White-Tailed Deer to Rift Valley Fever Virus
Center for Grain and Animal Health Research: Arthropod Borne Animal Disease Research Unit
Manhattan, Kansas
Rift Valley fever virus (RVFV), a zoonotic arbovirus, poses major health threats to livestock and humans if introduced into the United States. Although domestic cattle, sheep, and goats are susceptible to RVFV and function as amplification hosts during epidemics, the potential role of wildlife species such as white-tailed deer is unknown. Since white-tailed deer are abundant throughout the country, there is concern they could also serve as an amplifying host and become a reservoir and source of infection for livestock and humans. ARS scientists in Manhattan, Kansas, in collaboration with Kansas State University scientists, investigated the susceptibility of these deer to RVFV and confirmed their susceptibility to this virus. Importantly, infected deer developed hemorrhagic enteritis and bloody diarrhea, resulting in horizontal transmission to control animals. The results of this investigation provide evidence for a potentially major epidemiologic role for white-tailed deer if an outbreak of RVFV ever occurred in the United States.

Using Gene-Editing as a Tool to Engineer an African Swine Fever Vaccine
Plum Island Animal Disease Center: Foreign Animal Disease Research Unit
Orient Point, New York
African swine fever (ASF) is a deadly disease causing near 100% mortality in swine, trade restrictions and significant economic losses globally. The current threat for an introduction of ASF into the United States has never been higher. Since the introduction of ASF into the Republic of Georgia in 2007, 16 countries have reported new ASF

outbreaks, including Belgium and China in 2018. Currently there is no commercially available vaccine to prevent this devastating disease. ASF is a large and complex double stranded DNA virus. After more than 50 years of research, there are no efficient molecular tools available to help develop a safe and effective live recombinant ASF vaccine. ARS scientists in Orient Point, New York, investigated the use of the "CRISPR-Cas9" gene-editing system as a potentially more robust and efficient system to produce live recombinant ASF viruses. Gene editing is a new type of genetic engineering in which DNA can be directly inserted, deleted, modified or replaced in the genome of a living organism. Unlike early genetic engineering techniques that randomly inserts genetic material into a host genome, genome editing directs the modification to site specific location. Using the CRISPR-Cas9 system, a recombinant ASF virus was successfully developed in record time compared to the use of traditional genetic engineering techniques. These results demonstrate the potential advantage of using CRISPR/Cas9 over traditional methods and should significantly improve our ability to develop a first generation modified live ASF vaccine.

Identification of Avian Influenza Epidemiological Risk Factors of Wild Birds in Mexico

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

The risk of an introduction of avian influenza in North America by infected wild birds is significant. To identify epidemiological risk factors between poultry and synanthropic birds (wild birds living close to humans and farms), ARS researchers in Athens, Georgia, conducted field studies in collaboration with a team of scientists from Mexico. These studies evaluated the risk that synanthropic birds bring to poultry farms in a highly densely productive area of Mexico. The Altos de Jalisco region in west central Mexico is the location of the largest concentration of poultry farms. Recently, this region witnessed the emergence of low pathogenic H5N2 and the highly pathogenic H7N3 influenza viruses. As a result of these field studies in Mexico, 82 species of wild birds were identified, with some of these species linked to poultry farms. The highest ranked species corresponded to the Mexican Great-tailed Grackle and the Barn Swallow; making those potential hosts for disease transmission of pathogens in the wild bird-poultry interface in the region of Jalisco. The ability to demonstrate epidemiological connections between wildlife and poultry is important to understand the risk to the poultry industry from wild birds. Because of the proximity with the United States, these Mexican regions present a high risk of introduction of avian influenza through trade, wild birds and illegal transport of birds, and therefore pose a significant threat to the U.S. poultry industry.

Validation of Inactivation Methods for Brucella[1]

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

Select Agents are pathogens that the Department of Health and Human Services (HHS) and/or the U.S. Department of Agriculture (USDA) have deemed to pose a severe threat to public health and safety and are regulated through the Federal Select Agent Program (FESAP). The FESAP has emphasized the need to validate methods for inactivation of

[1] Research accomplishment applicable to both Biodefense and Zoonotic Bacterial Diseases

bacterial and viral agents, because of publicized failures involving Select Agent pathogens and the danger posed by improper disposal of these agents. ARS scientists at Ames, Iowa, tested several inactivation methods (heat, methanol, acetone, filtration, and formalin) under various conditions. These experiments demonstrated that 95C heating for 1 hour, 67 percent methanol for 5 days, or formalin treatment for 30 minutes were sufficient to prevent recovery of Brucella from spiked samples. Filtration of sera through a 0.22 um filter removed all viable Brucella from spiked samples. This published data is of great interest to regulatory and biosafety personnel, and the hundreds of laboratories that work with Select Agents. It provides validated procedures that are effective for inactivating Brucella from sera and tissue samples which can mitigate the risk of inadvertent release and ensure laboratory personnel safety.

Component 2: Antimicrobial Resistance

Identification of Co-infecting Bacterial Agents in Birds Infected with Newcastle Disease Viruses

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

Newcastle disease viruses are the most prevalent cause of respiratory disease on poultry farms worldwide, including the United States. To understand causes of Newcastle disease vaccine failure in the field, ARS researchers in Athens, Georgia, conducted random sequencing of nucleic acids obtained from poultry samples isolated in endemic countries. The outcome has been the identification of previously unrecognized infections with bacteria from the genus Ochrobactrum. The bacteria were isolated and determined to contain antibiotic resistance genes. The complete draft genome of a total of eight *Ochrobactrum* species bacteria has been published in two articles. The results of these studies demonstrated that Newcastle disease infection is often accompanied by bacterial infections, and in these cases, multidrug-resistant novel *Ochrobactrum* species strains were isolated from a pigeon, a duck, and chickens. This work is important because it demonstrates that additional factors may be associated with Newcastle disease in the field. Importantly, the discovery of previously unrecognized co-infections with novel multidrug-resistant bacteria highlights the importance of effective Newcastle disease vaccination programs to potentially prevent secondary bacterial infections that may lead to the use of antibiotics and the selection of the antimicrobial resistant bacteria.

Nanoparticles Improve Vaccines against Coccidiosis[2]

Beltsville Agricultural Research Center: Animal Parasitic Disease Laboratory
Beltsville, Maryland

Coccidiosis, a gut disease of poultry, costs U.S. producers $350 million annually due to poor weight gain in affected animals and the costs of treatment. It is also listed by the World Organization for Animal Health (OIE) as a high priority disease for which improved vaccines would significantly reduce the need for antibiotic administration. Current vaccines are comprised of low doses of highly infectious organisms and better vaccines are needed. ARS scientists in Beltsville, Maryland, discovered that attaching a

[2] Research accomplishment applicable to both Antimicrobial Resistance and Parasitic Diseases

protective vaccine antigen to nanoparticles significantly improved efficacy. Chickens given the vaccine by oral administration at hatch showed improved weight gain and feed conversion efficiency, as compared to chickens vaccinated with the same antigen but without nanoparticles. This technology may markedly improve the health and welfare of poultry flocks, reduce the costs of poultry production and reduce the use of antibiotics in poultry by decreasing the occurrence of concomitant bacterial infections.

Component 3: Zoonotic Bacterial Diseases

Validation of Inactivation Methods for Brucella

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

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Development of Culture Method to Study Leptospirosis in Animal Hosts

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

Leptospirosis is an important human and animal disease worldwide. The number of serious human cases of leptospirosis seen annually throughout the world is estimated at over one million, with a case fatality rate above 10%. The disease is maintained in soil, water and wildlife reservoirs and because there are many serotypes of this bacterium it is difficult to fully vaccinate against it. In cattle, leptospirosis results in abortion, stillbirth, premature birth and reproductive failure. The composition of cattle vaccines is based on studies that were performed 25 years ago and may not reflect the strains that are currently circulating. An additional challenge to developing improved vaccines against leptospires is that these bacteria are very difficult to grow under laboratory conditions, and do not express the same proteins normally found during natural infection which makes fully characterizing them challenging. ARS researchers at Ames, Iowa, developed a novel method for culturing leptospires that more closely mimics natural infection. This unique culture method resulted in expression of *Leptospira* proteins normally found during infection that are usually not expressed under laboratory conditions. These data provide

novel insights on colonization and persistence of infection in natural hosts and could lead to new detection and control strategies for this important human and animal pathogen.

Component 4: Respiratory Diseases

A Subunit Vaccine against Streptococcus suis in Swine

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

Streptococcus suis is a bacterium that is an important and common cause of disease in pigs and costs the swine industry millions in losses annually. It is also listed by the World Organization for Animal Health (OIE) as a high priority disease for which improved vaccines would significantly reduce the need for antibiotic administration. ARS researchers at Ames, Iowa, with collaborators from the University of Cambridge identified five candidate proteins of *S. suis* which were formulated into a vaccine with different adjuvants to help stimulate an immune response. The vaccine was found to be effective at preventing disease caused by *S. suis*. In addition, antiserum from the vaccinated pigs was reactive against whole *S. suis* bacteria of differing serotypes indicating a potential for cross-protection. These proteins are now being developed into a vaccine by a commercial company that can be used by swine producers to protect against this devastating and costly swine disease. This technology may markedly improve the health and welfare of pigs, reduce the costs of pork production and reduce the use of antibiotics in pigs by decreasing the occurrence of diseases caused by an important bacterial pathogen.

Mycoplasma ovipneumoniae Identified in a Widening Array of Animals

Animal Disease Research Unit
Pullman, Washington

Mycoplasma ovipneumoniae, currently believed to be the primary agent of pneumonia in bighorn sheep, has been described as having a host range restricted to *Caprinae* species (sheep, goats, musk ox) with domestic sheep and goats being implicated as the historic and continued source for introduction of this bacterium in bighorn sheep. This assumption has dictated grazing practices and policy implementation by other federal and state agencies. ARS researchers in Pullman, Washington, performed epidemiological studies and identified *M. ovipneumoniae* in the following numbers of tested animals: 21 of 421 (5 percent) of caribou, 9 of 362 (2.5 percent) of moose, 23 of 184 (12.5 percent) of Dall's (thinhorn) sheep, and 5 of 43 (12.5 percent) mountain goats. ARS scientists in Pullman, Washington, in collaboration with several veterinarians and a state agriculture department identified this bacterium in captive white-tailed and mule deer exhibiting respiratory illness, and in a bison (no history reported). Determining the true host range of this bacterium is of utmost importance to livestock, federal and state stakeholders, as current policies to restrict grazing and thereby interactions between domestic small ruminants and bighorn sheep will not fully address possible transmission routes and remain ineffective until all vectors are considered.

Significant Domestic Sheep Breed Differences in Nasal Shedding of Mycoplasma ovipneumoniae Identified

Animal Disease Research Unit
Pullman, Washington

Mycoplasma ovipneumoniae, carried by domestic sheep and goats has been implicated as an important pathogen in bighorn sheep that negatively impacts their population recovery. In an effort to develop *M. ovipneumoniae* control strategies for livestock producers, ARS scientists in Pullman, Washington, using quantitative polymerase chain reaction (qPCR) assays discovered a significant sheep breed difference in shedding of this bacterium. There was no/low shedding for 14.7 percent of Polypay; 27.2% of Rambouillet, and 10.2% of the Suffolk sheep and high shedding in 20.3 percent of Polypay; 9.6 percent of Rambouillet, and 28 percent Suffolk. The remainder of the tested sheep were intermittent high and low to no shedders. Based on this data, significantly fewer Rambouillet are high shedders of this bacterium as compared to Polypay and Suffolk breeds. The results support the hypothesis of a genetic association with carriage and shedding of *M. ovipneumoniae* and indicates mitigation of shedding may be obtained through selective breeding.

Component 5: Priority Production Diseases

None

Component 6: Parasitic Diseases

Nanoparticles Improve Vaccines against Coccidiosis

Beltsville Agricultural Research Center: Animal Parasitic Disease Laboratory
Beltsville, Maryland

Coccidiosis, a gut disease of poultry, costs U.S. producers $350 million annually due to poor weight gain in affected animals and the costs of treatment. It is also listed by the World Organization for Animal Health (OIE) as a high priority disease for which improved vaccines would significantly reduce the need for antibiotic administration. Current vaccines are comprised of low doses of highly infectious organisms and better vaccines are needed. ARS scientists in Beltsville, Maryland, discovered that attaching a protective vaccine antigen to nanoparticles significantly improved efficacy. Chickens given the vaccine by oral administration at hatch showed improved weight gain and feed conversion efficiency, as compared to chickens vaccinated with the same antigen but without nanoparticles. This technology may markedly improve the health and welfare of poultry flocks, reduce the costs of poultry production and reduce the use of antibiotics in poultry by decreasing the occurrence of concomitant bacterial infections.

A New Cure for Hookworm

Beltsville Agricultural Research Center: Animal Parasitic Disease Laboratory
Beltsville, Maryland

Hookworms are intestinal nematode parasites that infect nearly half a billion people globally. They cause iron-deficiency, anemia and productivity losses of up to $139 billion annually. In some places, available drugs have less than a 40% cure rate, increasing the threat posed by emerging drug resistance. Fortunately, a pore-forming protein (Cry5B) produced by the soil bacterium Bacillus thuringiensis (Bt) has demonstrated good efficacy against *Ancylostoma ceylanicum* hookworm infections in hamsters. ARS scientists at Beltsville, Maryland, broadened the application of Cry5B to dogs infected with *Ancylostoma caninum*, and hamsters as a model for human hookworm infection with *Necator americanus.* This protein, Cry5B, was shown to be highly effective against all hookworm parasites tested and can be improved by neutralizing the effects of stomach acids. Efficacy did not depend on the host immune system and did not decline with repeated dosing. A pan-hookworm therapy with excellent properties for use in humans and other animals has thereby been discovered.

Diagnostic Tests to Detect Horses Infected with Theileria-like Parasite

Animal Disease Research Unit
Pullman, Washington

During a 2009 equine piroplasmosis outbreak in Texas, ARS scientists in Pullman, Washington, discovered a new parasite of horses that resembles *Theileria equi* but is genetically distinct and undetectable by official diagnostic tests. The lack of detection methods to identify horses infected with this *Theileria*-like parasite may allow entrance of infected horses to the United States. ARS scientists identified potential genes and proteins that can be used for the development of diagnostic assays. These genes and proteins were tested to determine if they could be used in molecular or serologic assays to detect horses infected with the *Theileria*-like parasite. Results demonstrated that all horses infected with the *Theileria*-like parasite were consistently detected by these newly developed assays. This information will provide diagnostic assays that can be used by the Animal and Plant Health Inspection Service (APHIS) for the development of strategies to control the newly emerging pathogen.

Component 7: Transmissible Spongiform Encephalopathies

Identifying and Breeding Goats Resistant to Scrapie

Animal Disease Research Unit
Pullman, Washington

Scrapie is a fatal brain disease of goats and sheep for which there is no treatment. Scrapie is caused by the progressive accumulation of an abnormal form of the prion protein and loss of brain cells that often leads to abnormal behavior, lack of coordination, gait abnormalities and reduced mobility and body condition, but which always leads to death. Historically, a single diagnosis of scrapie results in permanent quarantine or euthanasia of all goats and sheep on the farm. The sheep industry has had an important tool in the struggle to eradicate scrapie in the form of genetic resistance. The strong resistance to

scrapie in sheep comes from the R171 gene allele, which changes the amino acid at position 171 of the prion protein to arginine (written simply as “R”). Conversely, genetic resistance has not been available to the goat industry. This recently changed with the discovery of two naturally occurring prion gene alleles in goats that have shown exceptional promise for conferring resistance. The first is S146, which denotes a serine (S) amino acid at prion protein position 146. The second is K222, which denotes a lysine (K) amino acid at position 222. Through an experiment that has lasted close to 10 years, ARS scientists in Pullman, Washington, along with other laboratories around the world have shown that even one copy of either S146 or K222 confers strong resistance to classical scrapie in goats, much like R171 in sheep. Although the USDA National Scrapie Eradication Program has not yet formally recognized these alleles, USDA is planning pilot genetic based cleanup plans for goats similar to what has been done for sheep. Scrapie resistance should enhance goat breeding programs and goat health in major ways. Importantly, breeding scrapie resistant goats will benefit all small ruminant producers by reducing scrapie in the United States and help work towards import/export status for the United States as a scrapie-free country.