Animal Health (NP 103) Annual Report for 2014

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. Cyril G. Gay and Eileen L. Thacker, National Program Leaders, Animal Health, manage the program.

The Animal Health National Program initiated the current five-year national program cycle Fiscal Year (FY) 2012. The Animal Health National Program currently includes 45 core research projects currently supported by 103 scientists located at 11 research sites throughout the country. The ARS research budget for the Animal Health Program FY 2014 was $68,044,045, increased from $61,917,200 FY 2013.

The following scientists in NP 103 received prominent awards in 2014:

ARS Hall of Fame

Dr. Hyun Lillehoj, Animal Biosciences and Biotechnology Laboratory (ABBL), Beltsville, Maryland, was inducted in the ARS Hall of Fame September 2014 for her lifelong accomplishments in avian immunology and research on alternatives to antibiotics for use in poultry production. Dr. Lillehoj was also selected by the American Association of Veterinary Immunologists as the winner of 2014 Distinguished Veterinary Immunologist.

William Hunting Award

Drs. Patti Miller and Claudio Afonso at the Southeast Poultry Research Laboratory (SEPRL), Athens, Georgia, along with research partners at PATH (Program for Appropriate Technology in Health), Seattle, Washington, and GALVMed (Global Alliance for Livestock Veterinary Medicines), United Kingdom, won the 2014 *William Hunting Award* for their paper "Development of a low-dose fast-dissolving table formulation of Newcastle disease vaccine for low-cost backyard poultry immunization." The journal *Veterinary Record* bestows this award annually to a paper they feel has made the most useful contribution to veterinary science that year.

American Association of Equine Practitioners (AAEP) Research Award
Dr. Don Knowles, Research Leader at the Animal Disease Reseach Unit (ADRU), Pullman, Washington, was awarded the first AAEP Research Award for his research on developing diagnostic and control strategies for equine piroplasmosis.

American Association of Veterinary Immunologists Elected Officers
Drs. Crystal Loving and Laura Miller, National Animal Disease Center (NADC), Ames, Iowa was elected President and President-Elect, respectively.

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

The sheep genome illuminates the biology of the rumen
ARS scientist Stephen White, Pullman, Washington, participated in the International Sheep Genomics Consortium with participants from a number of laboratories from around the world. Sheep (*Ovis aries*) are a major source of meat, milk, and fiber in the form of wool and represent a distinct class of animals that have a specialized digestive organ, the rumen, that carries out the initial digestion of plant material. The consortium assembled the sheep genome for the first time and used a large amount of gene expression data from 40 different tissues to show where on chromosomes the genes are located and where in the body they are active. This information was used to investigate several unique *Ovis aries* traits, including assessing how sheep process low-quality forage into high-quality animal protein. This project provides a reference sheep genome assembly that will form the basis of future research into all aspects of sheep biology, including susceptibility to infectious disease.

Porcine granulocyte-colony stimulating factor (G-CSF) delivered via replication-defective adenovirus induces a sustained increase in circulating peripheral blood neutrophils
The use of cytokines that stimulate the immune system as alternatives to antibiotics is a promising area for biotherapeutic use to prevent and combat infectious disease. ARS scientists at the National Animal Disease Center (NADC), Ames, Iowa, have investigated the potential value of using the granulocyte-colony stimulating factor (G-CSF) as a potential alternative to antibiotics in food-animal production as a possible candidate for pathogenic bacteria in which neutrophils (white blood cells that are the first line of defense against bacterial infections) can provide protection. G-CSF enhances the production and release of neutrophils from bone marrow and is already licensed for use in humans. A limitation of cytokines is their short half-life, which may limit their usefulness as a one-time injectable in production-animal medicine. ARS scientists found that the administration of recombinant G-CSF induced a transient increase in neutrophils (neutrophilia) in pigs; however, delivery of porcine G-CSF inserted in a replication-defective adenovirus (Ad5) vector significantly increased the neutrophilia pharmacodynamics effect. Pigs given one injection of the Ad5-G-CSF had a neutrophilia that peaked between days 3-11 post-treatment and neutrophil counts remained elevated

for more than 2 weeks. Neutrophils from Ad5-G-CSF treated pigs were fully functional based on laboratory tests, demonstrating that G-CSF may be an effective alternative to antibiotics for treating bacterial pathogens that are susceptible to neutrophils.

First validated genetic marker test for post-infection control of ovine progressive pneumonia virus

Ovine progressive pneumonia virus is a small ruminant lentivirus that causes long-term, progressively worsening pneumonia and mastitis in domestic sheep. Some sheep have a genetic predisposition to experience less severe disease from the virus, but there have been no specific genetic tests to predict which sheep these might be. ARS scientists in Pullman, Washington, and Dubois, Idaho, in collaboration with Washington State University, demonstrated that sheep with two copies of a small deletion near the ZNF389 gene were able to control viral replication. This result was observed in multiple sheep flocks under widely differing management and viral load conditions. This is the first validated genetic marker test for post-infection control of ovine progressive pneumonia virus, and it can be used to breed sheep with better ability to control the virus.

Potential virulence factor of Asian highly-pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV)

Asian highly-pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV), foreign to this country, is a serious threat to our nation's swine and agricultural economy. HP-PRRSV causes more severe disease than the PRRSV strains we have circulating in the United States, but we do not know why. ARS scientists at the National Animal Disease Center in Ames, Iowa, in collaboration with scientists at the University of Denver, examined the enzymatic activity of a small part of a viral protein, referred to as a protease. Proteases are enzymes that break down proteins. The scientists found that the HP-PRRSV region was 40 times more capable of cleaving specific types of a cellular protein called ubiquitin than that of a U.S. strain of PRRSV that causes only mild disease in pigs. Ubiquitin has been implicated in the regulation of many cellular processes, including the control of immune responses. The actions of this small part of the viral protein of HP-PRRSV may correlate with the increased disease seen and may serve as a target for vaccine design.

Development of a Rift Valley fever virus challenge model to evaluate vaccines in sheep and goats

Rift Valley fever virus (RVFV) is transmitted by mosquitoes and causes severe to fatal disease in ruminants and humans, which can be preventable by vaccination. Ruminants are known to amplify RVFV and are a potential source of infection for humans. Availability of a challenge model is a pre-requisite for vaccine efficacy trials. Several modes of inoculation were tested by ARS scientists at the Arthropod-Borne Animal Diseases Research Unit (ABADRU), Manhattan, Kansas, in collaboration with scientists at the National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, Manitoba, Canada. Differences in development of infections in sheep and goats were observed between animals inoculated with RVFV produced in mosquito cells compared to mammalian cells. Only RVFV produced in mosquito cells led to development of virus in the blood (viremia) in all inoculated animals. The insect cell-

produced RVFV appeared to be more infectious with earlier onset of viremia, especially in sheep, and may also more closely represent a field situation. These finding were used to develop a challenge protocol suitable for evaluating the efficacy of RVF vaccines in sheep and goats.

Amino acid changes in a viral protein determine the evolution of swine influenza A H3N2 viruses

Swine influenza A virus is an endemic and economically important pathogen in pigs with the potential to infect other host species including humans. Pigs may also become infected with human influenza A viruses. The viral hemagglutinin (HA) protein binds virus to cells and is the primary target of protective immune responses and the major component in swine influenza A vaccines. However, as a result of genetic mutations known as antigenic drift, vaccine virus strains must be regularly updated to reflect currently circulating strains. Characterizing how different virus strains in pigs are to the seasonal influenza virus strains in humans is also important in assessing the relative risk of interspecies transmission. ARS scientists at the National Animal Disease Center in Ames, Iowa, found that two primary swine influenza virus strains are currently circulating in the U.S. pig population, but with enough diversity between the HA proteins to suggest updates in vaccine strains are needed. ARS scientists identified specific changes in the HA protein that are likely responsible for differences between the two viruses. These changes may be useful in predicting when vaccines need to be updated. The differences between current seasonal influenza H3N2 strains in humans and those endemic in swine is enough that population immunity is unlikely to prevent the introduction of human viruses into pigs and vice-versa, reinforcing the need to continuously monitor and prepare for influenza A viruses.

Vaccinating against intestinal parasites

Anthelmintic resistance is a major problem in controlling parasites in production animals. Parasites produce proteins that modulate and suppress the host's immune responses providing an environment that is conducive to the parasite's survival. ARS scientists in Beltsville, Maryland, conducted a trial using a recombinant protein against the parasite, Ostertagia. The protein was used to vaccinate a number of animals, which had a high degree of protection against parasite infection and damage. Future studies on a larger number of animals are being planned. In addition to the protein used in these studies, additional potential vaccine candidates have been identified. Developing vaccines against parasites will help reduce the reliance on drugs that are becoming increasing ineffective in controlling parasites.

Identification of biomarkers for early diagnosis of Mycobacterium bovis

Mycobacterium bovis is the primary causative agent of tuberculosis (TB) in cattle. A program to eradicate bovine TB began in 1917, however eradication is elusive. Diagnosis of bovine TB remains problematic, especially in the early stages of the disease. Recent work by ARS scientists in Ames, Iowa, resulted in the identification of several potential biomarkers in the blood of infected cattle that may enable more accurate diagnosis of bovine TB. The development of effective blood tests will facilitate ease of detection and may potentially improve our ability to eradicate this zoonotic disease.

Virulence genes associated with Haemophilus parasuis infection
Haemophils parasuis causes Glasser's disease in pigs, characterized by chronic debilitation and death costing the swine industry millions annually. Currently there is no effective vaccine for *H. parasuis*. Research scientists in Ames, Iowa, had previously identified 10 strains of *H. parasuis* with differing levels of virulence. They recently sequenced the 10 strains and identified genes in some strains that may be associated with virulence and disease. These genetic areas can be used to develop vaccines that may be effective against this costly pathogen.

Evaluation of the Risk of Indigenous Ticks Transmitting Equine Piroplasmosis
Equine piroplasmosis was eradicated from the United States in the late 1980's. However, a recent outbreak in Texas caused significant economic loss to the equine industry and suggested that some ticks indigenous to the United States could play a role in transmission. ARS scientists in Pullman, Washington, in collaboration with Texas A&M University, collected and colonized ticks from horses at the outbreak ranch. The scientists demonstrated that these indigenous ticks were able to acquire and transmit the parasite to naïve horses. These results confirm that introduction of infected horses into areas of the United States containing competent indigenous vectors can result in dissemination of the parasite and thus disease to the equine population in the United States.