Animal Health (NP 103) Annual Report for 2015

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 (NPL), 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 42 core research projects currently supported by 100 scientists located at 11 research sites throughout the country. The ARS research budget for the Animal Health Program FY 2015 was $69 million.

The following scientists in NP 103 received prominent awards in 2015:

Service to America Medal
Dr. Hyun Soon Lillehoj, Animal Biosciences and Biotechnology Laboratory, Beltsville, Maryland, received the Career Achievement Medal, one of the Service to America Medals (also known as the "Sammies"). The Samuel J. Heyman Service to America Medals are presented annually by the nonprofit, nonpartisan Partnership for Public Service to celebrate excellence in the Federal civil service. Honorees are chosen based on their commitment and innovation, as well as the impact of their work on addressing the needs of the nation. The medals aim to extol excellence in our federal workforce and inspire other talented and dedicated individuals to go into public service.

2015 American Association of Avian Pathologists (AAAP) New Investigator Award
Dr. John Dunn of the Avian and Diseases and Oncology Laboratory, East Lansing, Michigan, received the 2015 Bayer New Investigator Award that is given each year by the American Association of Avian Pathologists to a scientist whose career as an independent investigator in poultry medicine began less than seven years ago and who has made meritorious research contributions to the avian research field.

In 2015 NP 103 scientists participated in research collaborations with scientists in:
Argentina, Australia, Austria, Belgium, Brazil, Cameroon, Canada, China, Colombia, Denmark, England, Egypt, Finland, France, Germany, India, Indonesia, Iraq, Ireland, Israel, Italy, Japan, Jordan, Kenya, Korea (South), Lithuania, Malawi, Mexico, Netherlands, New Zealand, Northern Ireland, Norway, Pakistan, Poland, Russia,

Scotland, South Africa, Spain, Sweden, Switzerland, Tanzania, Turkey, Uganda, Ukraine, United Kingdom and Vietnam

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

USDA speeds development of bird flu vaccine
The Agricultural Research Service (ARS) developed in record time an effective vaccine against the highly pathogenic avian influenza (HPAI) virus strains that caused the death of over 45 million chickens and turkeys in the United States. Although these HPAI viruses were not the cause of any human deaths, concerns remain that these viruses could mutate and become more harmful. Vaccines constitute a critical veterinary medical countermeasure to respond to biological threats such as avian influenza viruses. Currently, no vaccines for HPAI are licensed or permitted in the United States. The use of HPAI vaccines is dependent on our ability to rapidly develop vaccines with good efficacy against the virus strains that are the cause of the disease outbreak. In response to the first detections of new HPAI viruses (H5N8 and H5N2) in wild waterfowl and captive raptors in the United States in December 2014, ARS refocused its entire team of scientists working on avian influenza research to the most imminent research needs to address the U.S. outbreak, including the rapid development of a vaccine for emergency use. Within weeks, scientists at the Southeast Poultry Research Laboratory in Athens, Georgia, rapidly engineered a vaccine (rg-H5 vaccine) using reverse genetics technology that matches the H5N2 and H5N8 HPAI viruses that was the cause of the disease outbreak. The power of the reverse genetics technology is twofold. First, it allows the manipulation of the genes to change the hemagglutinin (HA) gene from its typical amino acids to having a sequence similar to other Low Pathogenic Avian Influenza (LPAI) viruses. This allows the change of a HPAI virus into a LPAI virus for safe production without affecting the efficacy of the vaccine virus. The second factor is that it allows the creation of a unique vaccine virus by swapping the HA gene with the one that matches the HA genes causing the disease outbreak. ARS with the support of the ARS Office of Technology Transfer and APHIS developed and implemented in record time a technology transfer plan that enabled the transfer of the rg-H5 vaccine to a commercial partner for development and production.

Novel vaccines effective against poultry diseases
Vaccination is one method used to help prevent the spread of infectious poultry diseases, but current vaccines could be safer and more effective. ARS scientists in Athens, Georgia, have developed vaccines to help reduce virulent and virus shed—excretion of virus by the host—and disease transmission from infected birds to healthy ones. This novel vaccine was shown to protect chickens against infectious laryngotracheitis virus (ILTV) and Newcastle disease virus (NDV), two of the most economically important infectious diseases of poultry. Both viruses cause sickness and death in domestic and commercial poultry as well as in some wild birds throughout the world. While current ILTV live-attenuated vaccines are effective, some of the viruses used to make them can

regain virulence—causing chickens to become chronically ill. Other types of vaccines can protect birds from the disease's clinical signs, but barely reduce the virus shedding in their respiratory secretions after infection. In that sense, those vaccines are not that effective, because they do not reduce the risk of virulent ILTV transmission to uninfected birds. With the support of the ARS Office of Technology Transfer, this novel vaccine is currently under development under a collaborative agreement with a commercial partner.

African swine fever virus experimental vaccine confers protection against a virulent virus challenge

African swine fever virus (ASFV) is the etiological agent of a contagious and often lethal disease of domestic pigs that has significant economic consequences for the swine industry. The control of African Swine Fever (ASF) has been hampered by the unavailability of vaccines. ASFV is one of the largest virus known, and the function of the large majority of the viral genes are unknown. Experimental vaccines have been developed using genetically modified-live attenuated ASFV where viral genes were removed from the genome. However, to date, none of these viruses have proven to be fully attenuated or effective. ARS scientists at the Plum Island Animal Disease Center have engineered a recombinant virus by specifically deleting six genes thought to be associated with virulence. Studies conducted in pigs showed that when this recombinant virus was inoculated in pigs, the virus was completely attenuated and did not cause disease. Importantly, when these animals were subsequently exposed to highly virulent ASFV strain, no signs of the disease were observed. This is the first report demonstrating the role of these genes acting as independent determinants of ASFV virulence. Additionally, this is the first experimental vaccine reported to induce protection when challenged against this very virulent strain.

Understanding the mechanisms that drive persistent infections in FMD-infected cattle

Tissues obtained post-mortem from cattle persistently infected with foot-and-mouth disease virus (FMDV) were analyzed by ARS scientists at the Plum Island Animal Disease Center to characterize the tissue-specific localization of FMDV and assess the expression of genes associated with the host immune response. Analysis of 28 distinct anatomic sites from 21 steers infected with FMDV had the highest prevalence of overall viral detection in the dorsal nasopharynx and dorsal soft palate. FMDV was less frequently detected in laryngeal mucosal tissues, oropharyngealmucosal sites, and lymph nodes draining the pharynx. Within persistently infected mucosal tissues, FMDV antigens were rarely detectable within few epithelial cells in regions of mucosa-associated lymphoid tissue. Assessment of the genes associated with the host immune response of persistently infected pharyngeal tissues, indicated a general trend of decreased gene expression for 14 genes compared to uninfected control animals. Overall, this study demonstrated that during the FMDV carrier state in cattle, viral persistence is associated with epithelial cells of the nasopharynx in the upper respiratory tract and decreased levels of expression of several genes associated with the immune response in the infected tissues.

Global Migration of Influenza A Viruses in Swine

The emergence of the 2009 A/H1N1 pandemic virus underscores the importance of understanding how influenza A viruses evolve in swine on a global scale. To reveal the frequency, patterns and drivers of the spread of swine influenza virus globally, ARS scientists at the National Animal Disease Center in Ames, Iowa, conducted the largest genetic analysis of swine influenza viruses undertaken to date, integrating demographic and swine trade data. Using genetic and modeling approaches, ARS scientists demonstrated the importance of the asymmetrical global live swine trade on the evolution of influenza virus diversity. The size of a country's swine population was not found to be an important independent factor, as exemplified by China, which hosts the world's largest swine population but has relatively little outgoing swine trade and does not appear to be a major source of viral diversity in neighboring Asian countries or globally. Rather, Japan, Thailand, Vietnam, and South Korea independently import influenza viruses from Europe and North America via long-distance live swine trade. As an extension of these observed patterns, ARS scientists built a population simulation model for the global spread of swine influenza viruses that incorporated trade data and could estimate the likelihood of emergence of the H1N1 pandemic virus in swine in the years leading up to 2009. ARS scientists found that the evolution of swine influenza viruses is most likely to occur in East and South-East Asia. Knowledge of the global linkages between swine influenza virus populations has important implications for designing efficient surveillance strategies in resource-limited settings and predicting future disease threats.

Discovering why some Avian Influenza vaccines fail

Vaccination is critical in protecting birds, animals, and people in developing countries like Indonesia where the H5N1 highly pathogenic avian influenza virus has become endemic. Indonesia has implemented a vaccination program, but some of its commercial vaccines have failed to protect poultry. An international collaboration of government officials, regulators, and scientists—including a team from the Southeast Poultry Research Laboratory in Athens, Georgia, investigated outbreaks in Indonesian flocks that were vaccinated. The team evaluated 14 Indonesian licensed vaccines to identify the seed strain—type of virus—included in the vaccines and variant field viruses to find out why these vaccines were failing. The results of this study showed that 11 of the 14 vaccines contained the manufacturer's listed vaccine seed strains, but 3 vaccines contained different seed strains than the ones listed on the label. Scientists immunized chickens with each of the 14 vaccines and found that protection varied greatly. Tests showed some vaccines contained a lot of antigens and some had only a little. The antigen, a protein from the virus, allows birds to produce antibodies to build up immunity. Vaccinated birds were challenged with three different field viruses. All vaccines protected against one of the viruses, some protected against the second virus, and none protected against the third. This research demonstrated the need to evaluate vaccines often and replace vaccine seed strains with more effective ones as new field viruses emerge that are resistant to older vaccines. Improving vaccines and ensuring they work not only helps control avian influenza outbreaks, but also adds new vaccines to the U.S. emergency stockpile.

Comparative analysis of the intestinal bacterial and viral communities from birds on chicken farms

There is a great deal of interest in characterizing the complex microbial communities in the poultry gut, and in understanding the effects of these dynamic communities on poultry performance, disease status, animal welfare, and microbes with human health significance. Field investigations undertaken by ARS scientists at the Southeast Poultry Research Laboratory to characterize viruses in the gut of poultry identified several novel poultry viruses, but the roles these viruses play in disease and performance problems have yet to be fully understood. The complex bacterial community present in the poultry gut influences gut development, immune status, and animal health, each of which can be an indicator of overall performance. Analysis of the intestinal viruses from birds placed on poultry farms revealed colonization by members of the *Picornaviridae*, *Picobirnaviridae*, *Reoviridae*, and *Astroviridae*. Analysis of the birds gut bacterial community revealed an altered community, notably by members of the *Lachnospiracea*/Clostridium and Lactobacillus families and genera. Members of the avian enteric *Reoviridae* and *Astroviridae* have been well-characterized and have historically been implicated in poultry enteric disease; members of the *Picobirnaviridae* and *Picornaviridae* have only relatively recently been described in the poultry and avian gut, and their roles in the recognized disease syndromes and in poultry performance in general have not been determined. This study has provided insight into the colonization of the poultry gut by enteric microbes circulating in commercial broiler flocks, and has identified enteric viruses and virus communities that warrant further study in order to understand their role(s) in avian gut health and disease.

Host-pathogen responses to Necrotic Enteritis in two inbreed chicken lines

Necrotic enteritis (NE) is an important intestinal infectious disease of commercial poultry flocks caused by *Clostridium perfringens*. Using an experimental model of NE, ARS scientists in Beltsville, Maryland, and East Lansing, Michigan, collaborated to identify the genetic mechanisms that might regulate the host response to this disease. Genomic tools were used to measure gene expression, resulting in the identification of 1,049 genes whose levels of expression were altered in intestinal lymphocytes from infected Ross chickens compared with uninfected controls. Five biological functions, all related to host immunity and inflammation, and 11 pathways were identified from this dataset. To further elucidate the role of host genetics in NE susceptibility, two inbred chicken lines, ADOL line 6 and line 7, which have identical major histocompatibility genes (enables immune cells to recognize pathogens) but differ in their susceptibility to virus infection, were compared for clinical symptoms and the expression levels of a panel of immune-related genes during experimental NE. Line 6 chickens were more susceptible to development of experimental NE compared with line 7. Of 21 immune-related genes examined, 15 were increased in infected line 6 versus line 7 chickens. These results suggest that immune pathways are activated in response to experimental NE infection and that genetic determinants outside of the chicken major histocompatibility genes influence resistance to this disease.

A genome-wide association study for the incidence of persistent bovine viral diarrhea virus infection in cattle

Bovine viral diarrhea viruses (BVDV) comprise a diverse group of viruses that cause disease in cattle. BVDV may establish both transient and persistent infections depending on the developmental stage of the animal at exposure. ARS scientists at the National Animal Disease Center in Ames, Iowa, conducted a study to determine whether genomic regions harboring genetic mutations called single nucleotide polymorphisms (SNP) could be associated with the presence or absence of persistent BVDV infection. A cattle genome-wide association approach based on 777,000 SNP markers was used. Samples from 2400 animals identified as positive or negative for the presence of BVDV in skin samples were used. One SNP marker chromosome 14 was found to be significantly associated with BVDV persistent infection. Fifteen SNP markers, residing on chromosomes 1, 2, 6, 8, 10, 15 and 18, were moderately associated with persistent BVDV infection. The function of the genes harboring these mutations provides leads in understanding the mechanisms involved in BVDV persistent infections.

Development of an improved vaccine evaluation protocol to compare the efficacy of Newcastle disease vaccines

ARS scientists at the Southeast Poultry Research Laboratory developed an improved animal challenge model to evaluate the efficacy of Newcastle disease vaccines. This challenge model is more stringent than currently used models in that it allows the detection of statistical significant differences in mortality between Newcastle disease vaccines. This is important because different areas of the world are affected by different strains of Newcastle Disease virus and this system may improve the ability of veterinarians to choose a more appropriate vaccine for their region. This animal model also addresses the importance of evaluating vaccines for suppression of viral shedding. Current vaccine evaluation protocols do not evaluate the capacity of vaccines to reduce viral shedding. This deficiency is a problem in countries that do not practice culling (endemic poor countries) as the virus continues to be maintained in poorly vaccinated poultry farms.

Improved diagnosis of TSEs of sheep, goats, and elk

Gold standard diagnostic testing for the TSEs of small and wild ruminants in the United States is performed by immunohistochemistry analysis of formalin fixed tissues using an automated, monoclonal antibody based system with reagents and procedures developed by the Animal Disease Research Unit in Pullman, Washington. ARS researchers in Pullman, Washington, and their partners at Washington State University demonstrated that infectious prions can be detected from much smaller blood sample volumes, even during preclinical infection. This study supports further development of a safe and highly efficient blood-based diagnostic test for preclinical scrapie infection in sheep.

Increase in sensitivity and specificity of bovine tuberculosis diagnostic testing

A new serological diagnostic test for tuberculosis in cattle was licensed by the Center for Veterinary Biologics (CVB) in 2013 and approved for use in the bovine tuberculosis eradication program by USDA APHIS. Research during the fiscal year 2014 showed that the novel serological test was not accurate enough to entirely replace the current testing

method (tuberculin skin test). However, it was noted that tuberculin skin testing resulted in a "boosting effect" and an increase in the antibodies detected by the new serological test. The boost in antibody production resulted in an enhanced ability of the new serological test to detect *Mycobacterium bovis* infected cattle (increased accuracy). The two tests used together in this fashion, were better at identifying infected cattle than either test alone. A new paradigm was proposed to administer the tuberculin skin test and then draw blood for the new serological test during the boosting period. However, it was not clear when the boosting period began or ended. In the fiscal year 2015, ARS researchers in Ames, Iowa, determined both the beginning (7-10 days) and the ending (60-70 days) of the boosting period. This work allows APHIS TB program staff to direct field veterinarians to schedule testing at times that optimize the accuracy of testing, making the diagnosis of bovine tuberculosis more efficient and cost effective for both producers and USDA. This work provides a scientific basis for a change in USDA APHIS regulations directing field veterinarians to obtain blood for the novel test 7 to 70 days after skin testing.

Booster vaccination of adult cattle increases protection against virulent Brucella abortus

The high prevalence of brucellosis in free-ranging wildlife in the Greater Yellowstone Area (areas surrounding Yellowstone National Park) pose a risk for reintroduction of the disease to cattle. Over the last 15 years, approximately 25 cattle herds have been infected with brucellosis in the Greater Yellowstone Area. In collaboration with scientists from the University of Wyoming, ARS researchers in Ames, Iowa, evaluated the efficacy of single, double, or triple vaccinations with RB51 and compared protection against experimental challenge to animals that had not been vaccinated (controls). Data indicates that booster vaccination of cattle prevented abortion and reduced infection with animals receiving two or three vaccinations demonstrating greater protection. This study demonstrated that booster vaccination of cattle with RB51 will provide a high degree of protection against brucellosis and be an effective strategy for implementation in the brucellosis eradication program.

Development of an in vitro drug-sensitivity test for Eimeria

Medication of poultry feed with ionophore drugs or synthetic chemicals represents a major way to control avian coccidiosis in the poultry industry. However, this approach has become less efficacious because of drug-resistance in Eimeria parasites. While there are several different anti-coccidial drugs available, it is impossible to know ahead of time which drug to use on a particular farm because there are no rapid tests for estimating drug-sensitivity in the resident Eimeria population. ARS researchers developed an in vitro cell culture assay that utilizes chicken cells inoculated with the parasite in the presence or absence of ionophore drugs. The effect of these drugs on parasite invasion and development was measured by using microscopy or molecular methods which indicate that in vitro cell culture constitutes a viable, rapid, and less costly alternative to evaluating drug-sensitivity a broiler house. Poultry companies and poultry farmers will benefit from this technology by knowing which anti-coccidial drugs to use to prevent avian coccidiosis outbreaks.

Climatological variation and ecological perturbation drive geographic and host colonization events for parasites and pathogens

ARS researchers, in collaboration with academic scientists, characterized how episodic shifts in climate and environmental conditions combine with host switching and other ecological mechanisms to hasten parasite diversification. This view runs counter to more than a century of co-evolutionary thinking about the nature of complex host-parasite assemblages. The Animal Parasitic Diseases Laboratory introduced the concepts of hard tipping points and shifting balances (related to temperature thresholds for parasite development) to explain how the range expansion and establishment likely occurs. These new insights provide a framework to understand and predict how ongoing and accelerating climate change will influence the distribution of parasites and the emergence of disease in wild and domestic animals, with consequences for food sustainability, availability, and animal and human health.