National Program 103 Animal Health
National Program Annual Report: FY2010

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, are currently managing the program.

The Animal Health National Program initiated the current five-year national program cycle Fiscal Year (FY) 2007. The Animal Health National Program currently includes 45 core research projects supported by 100 scientists located at 11 research sites throughout the country. The ARS research budget for the Animal Health Program FY 2010 was $61,552,820.

The following scientists in NP 103 received prominent awards in 2010:

- **Joan K. Lunney**, Beltsville, Maryland, received the ARS Area Senior Research Scientist of the Year for significant research contributions and international leadership in determining immune mechanisms and genetic resistance for infectious pathogens of importance to the U.S. swine industry.

- **Amy L. Vincent**, Ames Iowa, received the ARS Area Early Career Research Scientist of the Year for excellence in swine influenza virus pathogenesis, transmission, and vaccine research supporting the U.S. swine industry.

- The ARS H1N1 Flu Virus Research Team, which includes scientists from the ARS Virus and Prion Research Unit, Ames, Iowa: **Amy Vincent, Kelly Lager, Kay Faabert, and Marcus Kehrli** and scientists from the ARS Exotic and Emerging Avian Viral Diseases Research Unit, Athens, Georgia: **Erica Spackman and David Suarez** as well as **Cyril Gay** and **Steven Kappes** from the ARS Office of National Programs, Beltsville, Maryland, received the "Special Administrator's Award" for outstanding rapid research support and technology development to assist USDA, cooperating agencies, and the U.S. pork industry respond to the 2009 H1N1 pandemic flu threat.

Scientists within the National Animal Health Program were very active in their fields during fiscal year 2010 with 225 articles published in peer-reviewed scientific journals. Many of the discoveries and findings were published in the popular press to reach our customers and stakeholders, including 15 articles in trade journals and book chapters.

Technology transfer activities for the National Animal Health Program included 15 invention disclosures, 8 new Cooperative Research and Development Agreements (CRADA), 35 active Specific Cooperative Agreements (SCA), and 96 Material Transfer Agreements (MTA).

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

Discovering the primary site of Foot-and-Mouth Disease Virus (FMDV) replication in cattle
It is well-established that the respiratory tract is the most important route of infection of FMDV in cattle. However, conflicting data from different research groups have implicated regions of either upper respiratory tract (nasopharynx) or lower respiratory tract (lungs) as the primary sites of infection. Recent work by scientists at the Plum Island Animal Disease Center has demonstrated that after aerosol exposure to the virus, the events that lead to infection and disease involve replication of FMDV in cells of the pharynx, followed by replication in lung, which coincides with the establishment of virus in the blood. This infection model demonstrated that massive viral amplification occurs in the lungs (with associated shedding to the environment) prior to appearance of the first lesions (vesicles) in the feet and mouth. The virus is established in the blood coincidently with further viral amplification in the lungs and at vesicle predilection sites. This scientific information is critical to the development of Foot-and-Mouth Disease countermeasures such as vaccines, which clearly will be needed to prevent FMDV from replicating in the pharynx and lungs in order to be effective. Based on this information, it is speculated that enhancement of mucosal immunity in the pharynx and lungs has a high probability of improving vaccine protection. Once the virus is established in the blood, the battle has already been lost. Continued efforts to improve the understanding of virus host interactions during early phases of infection will greatly contribute to the development of effective tools to block viral infection.

Determining the threat of Rift Valley Fever to the United States
To determine which arthropods should be targeted for control should Rift Valley Fever virus (RVFV) be detected in North America, ARS scientists at the Anthropod-Borne Animal Disease Research Unit in Manhattan, Kansas, in collaboration with the U.S. Army scientists at Fort Dietrick, Maryland, evaluated mosquito species from the western, midwestern, and southern United States for their ability to transmit RVFV. *Culex tarsalis* mosquitoes transmitted RVFV efficiently. In contrast, when exposed to the same virus dose, none of the other *Culex* species tested transmitted RVFV efficiently. Although *Aedes vexans* mosquitoes from Florida and Louisiana were relatively efficient vectors (transmitters) of RVFV, specimens of this species captured in Colorado or California were virtually incompetent, illustrating the need to evaluate local insect populations for their ability to transmit a pathogen. This is the first comprehensive vector competence study of U.S. mosquitoes for RVFV and provides key targets for vector control should an outbreak of RVFV ever occur in the United States.

Efficacy of Swine Influenza Vaccines Against the H1N1 Pandemic

The gene constellation of the 2009 pandemic A/H1N1 virus is a unique combination from swine influenza A viruses (SIV) of North American and Eurasian lineages, but prior to April 2009 this new and emerging virus had never before been identified in swine or other species. Although its hemagglutinin gene is related to a North American H1 SIV, it is unknown if vaccines currently used in U.S. swine would cross-protect against infection with the pandemic A/H1N1. ARS scientists at the National Animal Disease Center in Ames, Iowa, evaluated the efficacy of inactivated vaccines prepared with North American swine influenza viruses as well as an experimental homologous A/H1N1 vaccine to prevent infection and disease from 2009 pandemic A/H1N1. All vaccines tested provided partial protection ranging from reduction of pneumonia lesions to significant reduction in virus replication in the lung and nose. The multivalent vaccines demonstrated partial protection; however, none were able to prevent all nasal shedding or clinical disease. An experimental homologous 2009 A/H1N1 monovalent vaccine provided optimal protection with no virus detected from nose or lung at any time point in addition to amelioration of clinical disease. Based on cross-protection demonstrated with the vaccines evaluated in this study, the U.S. swine herd likely has significant immunity to the 2009 A/H1N1 from prior vaccination or natural exposure. However, consideration should be given for development of monovalent homologous vaccines to best protect the swine population thus limiting shedding and the potential transmission of 2009 A/H1N1 from pigs to people.

Direct-Fed Microbials Improve the Gut Health of Poultry

Probiotics modulate gut immunity and enhance natural resistance against avian coccidiosis. Direct-fed microbials (DFMs) are live microorganisms which confers a health benefit on the host by balancing its intestinal microbes. Recently, much attention has been paid to the role of DFMs on the immune system (i.e., immunomodulation) and their effects on the interaction between gut microflora and host immune system development. In order to develop a novel control strategy for poultry diseases and to reduce antibiotics use, ARS scientists in Beltsville, Maryland, investigated the immune mechanisms and possible immune enhancement using various direct-fed microbial products. Feeding dietary DFMs significantly improved intestinal structure and enhanced gut health as revealed by increased villus height and crypt depth compared with normal controls. These studies provide a rational scientific basis for future studies to investigate DFMs as immunopotentiating agents to enhance host protective immunity against enteric pathogens in broilers chickens.

Survey of Cattle Parasites and Resistance to Drug Therapy

A national survey of cattle intestinal parasites and their response to antiparasiticide drug (anthelmintic) treatment was conducted by ARS scientists in Beltsville, Maryland, in collaboration with the Animal Plant Health Inspection Service and two University collaborators. The results of the study of randomly selected cattle operations demonstrated a wide distribution of resistance to anthelmintic treatment. In nearly all cases, the species of parasite was *Cooperia* species, in particular *Cooperia punctata*.

While historically this parasite has been a minor species infecting cattle, the emergence of resistance to antiparasiticide treatment has resulted in it becoming a dominant pathogen. These results demonstrate that overuse of anthelmintics has not only selected for drug resistant intestinal parasites, but has also changed the population dynamics of parasites on pasture and selected for a species with increased ability to cause damage to the host. These results clearly demonstrate that there has been a rapid rise in the prevalence of cattle gastrointestinal parasites that are resistant to some of the most commonly used anthelmintics. Research is underway to evaluate the mechanism of anthelmintic resistance and to evaluate genetic changes in the host and parasite that may impact parasite control.

Diagnostics and Vaccine Evaluation for Brucella suis Infection in Cattle

The attempt to eliminate *Brucella abortus* from cattle in the United States has been the result of a national eradication program that began in 1934 costing more than $10 million. Although *B. abortus* has been largely eliminated from domestic cattle, the prevalence of *B. suis* in feral swine has emerged as a significant problem for domestic cattle. Cattle infected with *B. suis* test seropositive for brucellosis and antibody responses cannot differentiate between *B. suis* or *B. abortus*. Brucellosis is a regulatory issue and can lead to the loss of a state's brucellosis-free status and high economic costs to producers and state regulatory agencies. At this time, the time course for detecting and the development of antibodies to *B. suis* in acutely infected cattle is unknown. While vaccination for *B. abortus* using the vaccine RB51 is still commonly done in domestic cattle, the efficacy of RB51 vaccination in protecting cattle against *B. suis* infection is unknown. In this study the serologic responses of cattle to *B. suis* infection, lesions, and the tissue localization of *B. suis* in pregnant RB51-vaccinated and control cattle after experimental challenge was assessed. ARS scientists in Ames, Iowa, found that there was variation in the ability of various brucellosis diagnostic tests to detect antibodies in cattle infected with *B. suis.* Although an experimental challenge with *B. suis* did not cause abortions in cattle, there was an increased incidence of retained placentas. There was a high predilection for localization of *B. suis* in the mammary gland with shedding in milk increasing the potential of transmission to humans. In addition, cattle vaccinated with RB51 were not protected against *B. suis* infection. Research is ongoing to further develop diagnostic assays and vaccine strategies against *B. suis* in swine and cattle.

Persistence of Mycobacterium bovis in Vaccinated White –Tailed Deer

Programs for eradication of bovine tuberculosis caused by *Mycobacterium bovis* from the U.S. cattle were initiated in 1917 due to public health concern of bovine tuberculosis as a zoonotic disease. Traditional test and slaughter policies have been effective in lowering the prevalence of disease, but the incidence of new cases has been increasing and efforts to eradicate bovine tuberculosis from the United States have been frustrating. A current problem impeding eradication of bovine tuberculosis from the United States is the persistence of *M. bovis* infection in free-ranging white-tailed deer (WTD). This wildlife reservoir poses a serious threat to the bovine tuberculosis eradication effort. Other countries with established wildlife reservoirs have been unable to eradicate tuberculosis from domestic livestock and have abandoned eradication for disease control measures. The Michigan Departments of Agriculture and Natural Resources and Animal Plant

Health Inspection Service, Veterinary Services have indicated that vaccines may be critical for the control of bovine tuberculosis (TB) in Michigan (i.e., the TB core area). ARS researchers at Ames, Iowa, previously demonstrated that *M. bovis* bacillus Calmette-Guerin (BCG) used as a vaccine reduced disease severity in WTD upon experimental challenge with virulent *M. bovis*. In an extension of these studies, it was demonstrated that *M. bovis* BCG persists in tissues of WTD for up to 9 months after vaccination. The attenuated live vaccine was primarily detected in lymphoid tissues without evidence of colonization of muscle (i.e., meat potentially consumed by humans); thus, the risk of transmission to humans is minimal. These findings underscore the necessity of continuing detailed safety studies for use of vaccines intended for wildlife that may be consumed by humans.

Elimination and Control of Babesia caballi (Piroplasmosis) in Infected Horse

Equine piroplasmosis is a disease caused by blood parasites of the babesia family. It is considered a foreign animal disease in the United States and every effort has been made to prevent its entry into the U.S. horse population. The presence of this organism will prove very expensive to the equine industry due to blocking of export and importation of horses. During 2010 the United States encountered the reemergence of babesiosis, also known as piroplasmosis in the equine population. Babesiosis in horses is caused by two distinct parasites, *Babesia equi* and *Babesia caballi*. In order to begin developing strategies to control and re-eliminate the organism from the U.S. equine population, and in response to the needs of the Animal Plant Health Inspection Service, ARS scientists in Pullman, Washington, developed a method using imidocarb to eliminate persistent infection and transmission risk from horses infected with *Babesia caballi.* This has proven critical to the equine industry as it has resulted in owners of infected horses being able to treat their horses thus enabling them to resume their prior functions. In contrast, the second parasite *Babesia equi*, has proven more difficult to clear from infected horses. Research by ARS scientists has resulted in the sequencing and annotating of the *B. equi* genome discovering that this parasite is taxonomically between other Babesia organisms and a different blood borne parasite, Thieleria. The genetic information also determined that *B. equi* lacks a gene family that produces a classical antigenic variation. This discovery will enable ARS scientists to begin further research exploring and developing alternative intervention strategies to control this foreign disease entity in the U.S. equine population.

Genetic Differences between Non-virulent and Virulent Haemophilus parasuis Isolates

Respiratory disease remains one of the most important causes of disease to the swine industry. *Haemophilus parasuis* is a bacterium that causes Glässer's disease in swine, a disease characterized by chronic debilitation and often death that costs the swine industry millions in losses annually. However, not all strains of the bacterium cause disease. To date, little is known about genetic differences among *H. parasuis* strains and genetic factors that contribute to its ability to cause disease. Currently, there are no efficacious vaccines for *H. parasuis* and most control strategies have been poorly successful in preventing disease and the resulting loss of pigs. ARS scientists at the National Animal Disease Center in Ames, Iowa, compared four isolates of the bacterium for their ability to cause disease in pigs. Three of the isolates caused disease in the pigs, while pigs given

the fourth isolate remained healthy. Pigs that were given the non-disease causing isolate were subsequently protected from disease when exposed to one of the disease causing isolates. Thus, strains of *H. parasuis* that don't cause disease may be useful as vaccines to protect pigs against disease causing strains. In order to better understand the differences between *H. parasuis* isolates and potentially identify the mechanism by which some isolates fail to cause disease, DNA sequencing is being used to compare the genomes of the different isolates. These results will provide information so scientists can develop control strategies and potentially identify vaccine candidates that can be used by swine producers to control losses from this disease and identify whether strains of H. parasuis circulating on the farm will cause disease.