Animal Health (NP 103) Annual Report for 2011

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 U.S. 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) 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 2011 was $66,683,000.

The following scientists in NP 103 received prominent awards in 2011:

Marcus E. Kehrli, Jr., Supervisory Veterinary Medical Officer, Virus and Prion Diseases Management Unit, National Animal Disease Center (NADC), Ames, Iowa, received the Area Senior Scientist Award for "For sustained excellence in animal health research contributions and leadership."

Hyun Lillehoj, Research Molecular Biologist, Animal Parasitic Diseases Laboratory, Beltsville, Maryland, was awarded the 2011 Phibro Animal Health Excellence in Poultry Research Award by the American Association of Avian Pathologists (AAAP). This award is given to a member of AAAP for sustained excellence in research in poultry disease and health over a period of at least twenty years.

Michael Day, Microbiologist, Southeast Poultry Research Laboratory (SEPRL), Athens, Georgia, received the Bayer-Snoeyenbos New Investigator Award at the annual AVMA American Association of Avian Pathologists (AAAP) meeting in St. Louis, Missouri. This award is given to a member or associate member of AAAP, whose career as an independent investigator in poultry medicine began less than seven years ago and who during this time has made meritorious research contributions to the avian field.

Hans Cheng, Research Geneticist, Avian Disease and Oncology Laboratory, East Lansing, Michigan, received the 2011 Embrex Fundamental Research Award given by the Poultry Science Association at the 100th Annual Poultry Science Association Meeting in St. Louis, Missouri.

Amy L. Vincent, Veterinary Medical Officer, Virus and Prion Diseases Management Unit, National Animal Disease Center (NADC), Ames, Iowa, received the ARS Area Early Career Research Scientist award for excellence in conducting swine influenza research supporting the U.S. swine industry.

David Suarez and **Erica Spackman**, Exotic and Emerging Avian Viral Diseases Research Unit, Southeast Poultry Research Laboratory, Athens, Georgia, received the ARS Technology Transfer Award for their contribution to the H1N1 Pandemic Influenza Veterinary Diagnostic Test Development Team for timely development and transferral of H1N1 pandemic influenza diagnostic tests to the National Veterinary Services Laboratory.

Eduardo Casas, Supervisory Veterinary Medical Officer, ARS Ruminant Diseases and Immunology Research Unit, Ames, Iowa, received ASAS' Bouffault International Animal Agriculture Award for distinguished service to animal agriculture that helped developing countries.

Robert Silva, Research Microbiologist, Avian Disease and Oncology Laboratory, East Lansing, Michigan, for being Awarded the 2011 American Association of Avian Pathologists P. Levine Award for the Best Paper published in the journal, *Avian Diseases*, in 2010.

Anthony V. Capuco, Research Physiologist in the Bovine Functional Genomics Laboratory, Beltsville, Maryland, was named a Fellow of the American Dairy Science Association for his research contributions to the dairy industry and for his service to the Association. Dr. Capuco was presented with this award at the 2011 Joint Annual Meeting of the American Dairy Science Association and the American Society of Animal Science, held in New Orleans, Louisiana.

Jitender P. Dubey, Microbiologist, Animal Parasitic Diseases Laboratory, located in the Animal and Natural Resources Institute in Beltsville, Maryland, was elected as a member of the National Academy of Sciences on April 27, 2010, in recognition of his distinguished and continuing achievements in original research. Election to the Academy is considered one of the highest honors that can be accorded a U.S. scientist or engineer.

Jitender P. Dubey, Microbiologist, Animal Parasitic Diseases Laboratory, located in the Animal and Natural Resources Institute in Beltsville, Maryland, was elected to the Fellowship in the American Academy of Microbiology on May 24, 2011. The Academy, the honorific group within the American Society of Microbiology, recognizes excellence, originality, and creativity in the microbiological sciences.

Joan K. Lunney, Research Chemist at the Animal Parasitic Diseases Laboratory, located in the Animal and Natural Resources Institute in Beltsville, Maryland, received the Scientist of the Year Award 2011, for significant research contributions and international leadership in determining protective immune mechanisms and genetic resistance for infectious pathogens of importance to the U.S. swine industry.

Don Knowles, Research Leader at Animal Diseases Research Unit in Pullman, Washington, received the 2011 Sheep Industry Camptender Award and the 2011 Outreach and Diversity Award from ARS.

Scientists within the National Animal Health Program were very active in their fields during FY 2011, with 236 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 articles in trade journals and book chapters. Technology transfer activities for the National Animal Health Program included 17 invention disclosures, 17 new Cooperative Research and Development Agreements (CRADA), 71 active Specific Cooperative Agreements (SCA), and 8 new 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.

New Viruses Detected in Turkey Gut
The discovery of novel viruses in turkeys may help veterinarians unravel some of the mysteries of viral enteric diseases that affect poultry. Each year, enteric disorders such as Poult Enteritis Mortality Syndrome (PEMS) in young turkeys and Runting Stunting Syndrome (RSS) in chickens cause tremendous economic losses to the poultry industry worldwide due to increased mortality rates, decreased weight gain, and treatment costs. Decades of research indicate that certain viruses may be the culprit for viral enteric diseases, but no single agent has been identified. ARS scientists in Athens, Georgia, used a new powerful tool called "Metagenomics" to detect and sequence nucleic acid of all the RNA (ribonucleic acid) viruses present in the gut of turkeys affected by enteric syndromes. Metagenomics, a molecular technique, is the study of a collection of genetic material from a mixed community of organisms. The technology allows scientists to look at a complex environmental sample, sequence all the viral nucleic acid in the sample, and analyze it. ARS scientists extracted and analyzed nucleic acid from poultry intestine samples collected from five different turkey flocks affected by enteric diseases. The intestinal virus metagenome contained thousands of pieces of nucleic acid representing many groups of known and previously unknown turkey viruses. As suspected, avian viruses such as Astrovirus, Reovirus, and Rotavirus—common in the gut of birds and implicated in some enteric diseases—were verified. The detection of numerous small, round RNA viruses, such as the members of the Picornaviridae family, long thought to be a major constituent in the turkey gut, also was confirmed. However, ARS scientists found many previously unknown turkey viruses such as picobirnavirus, a small double-

stranded RNA virus implicated in enteric disease in other agricultural animals. A calicivirus also was identified in poultry for the first time. Caliciviruses are found in different animals and have been implicated for years in enteric diseases in humans. Discovering this treasure trove of virus sequences puts researchers a step closer to understanding viral communities in poultry, and will help scientists determine which viruses are associated with enteric diseases and which organisms are not.

DNA Vaccination Elicits Protective Immune Responses against Pandemic and Classic Swine Influenza Viruses in Pigs

Swine influenza is a highly contagious viral infection in pigs that significantly impacts the pork industry due to weight loss and secondary infections. There is also the potential of a significant threat to public health, as was seen in 2009 when the pandemic H1N1 influenza virus strain emerged from reassortment events among avian, swine, and human influenza viruses within pigs. As classic and pandemic H1N1 strains now circulate in swine, an effective vaccine may be the best strategy to protect the pork industry and public health. Current inactivated-virus vaccines available for swine influenza protect only against viral strains closely related to the vaccine strain, and egg-based production of these vaccines is insufficient to respond to large outbreaks. DNA vaccines are a promising alternative since they can potentially induce broad-based protection with more efficient production methods. ARS scientists in Ames, Iowa, working together with scientists at the National Institute for Health, (NIH) in Bethesda, Maryland, evaluated the potential of monovalent and trivalent DNA vaccine constructs to elicit immunological responses and protect pigs against viral shedding and lung disease after challenge with pandemic H1N1 or classic swine H1N1 influenza virus. Scientists also compared the efficiency of a needle-free vaccine delivery method to that of a conventional needle/syringe injection. The results of these studies demonstrated that DNA vaccination elicits robust serum antibody and cellular responses after three immunizations and confers significant protection against influenza virus challenge. Needle-free delivery elicited improved antibody responses with the same efficiency as conventional injection and may be considered for development as a practical alternative for vaccine administration.

Effects of Glycosylation on Antigenicity and Immunogenicity of Classical Swine Fever Virus Envelope Proteins.

Classical swine fever virus (CSFV) harbors three envelope glycoproteins (Erns, E1 and E2). Previous studies have demonstrated that removal of specific glycosylation sites within these proteins yielded attenuated and immunogenic CSFV mutant vaccine strains. ARS scientists in Orient Point, New York, in collaboration with scientists at the University of Connecticut, analyzed the effects of removing the glycosylation sites of the Erns, E1, and E2 proteins on immunogenicity. Interestingly, Erns, E1, and E2 proteins lacking glycosylation failed to induce a detectable virus neutralizing antibody response and protection against CSFV. Similarly, no neutralizing antibody or protection was observed in pigs immunized with E1 glycoprotein. Analysis of Erns and E2 proteins with single site glycosylation mutations revealed detectable antibody responses, but not protection against lethal CSFV challenge which is affected by removal of specific glycosylation sites. In addition, it was observed that single administration of purified Erns

glycoprotein induced an effective protection against CSFV infection. This discovery has important implications for the manufacturing and future development of CSF vaccines, demonstrating that complete deglycosylation of E2 and Erns erase completely their immunogenicity in swine. Additionally, this is the first report indicating that Erns can be immunogenic and induce protection by itself.

A Genetically-Engineered Swine Influenza Vaccine Confers Cross-Protection against Emerging Variant Virus Strains

It is widely recognized that the diversity of swine influenza virus (SIV) strains impedes the effective immunization of swine herds. This is of great concern as emerging variant swine influenza viruses could emerge into the human population. New variant viruses may also have significant negative economic impact on the swine industry. Therefore, the evaluation of modern vaccine technologies for SIV in the swine host is important for achieving greater control of emerging variant virus strains in swine populations and limiting the risk of transmission to humans. Live virus vaccines are considered to be more effective than inactivated or non-replicating virus vaccines as inducers of cellular immunity, but all licensed SIV vaccines in the United States are based on inactivated virus antigens. ARS scientists in Ames, Iowa, used molecular approaches to construct mutated H3N2 SIV genomes that result in attenuated replication properties. Truncation of a key viral protein (NS1) used by influenza virus to evade the host immune system produced a mutant virus with restricted replication in the swine respiratory tract but strong immunogenic properties. Intranasal inoculation of pigs with this virus resulted in robust protection against homologous challenge and significantly reduced viral replication and clinical signs upon challenge with a heterologous H1N1 SIV strain. ARS scientists demonstrated that cross-protection was mediated by the cell-mediated immune response.

Probiotics-mediated Immunomodulation of Poultry Innate Immunity

Probiotics or direct-fed microbials (DFM) are live microorganisms that provide alternatives to antibiotics. They are also known to confer health benefits on the host by influencing the host immune system via increased antibody production, up-regulation of cell-mediated immunity, and augmenting innate defense mechanisms. ARS scientists in Beltsville, Maryland, examined the role of *Bacillus subtilis*-based DFMs on macrophage functions such as nitric oxide production and phagocytosis, the two most important innate immune functions of macrophages. Macrophage, a key component of host innate immunity, participates in host defense by secreting cytokines and nitric oxide, which modulates inflammation and kills microbes. In controlled studies, ARS scientists demonstrated that certain strains of *Bacillus subtilis* increase macrophage function in broiler chickens. These studies provide the scientific basis for future studies to investigate DFMs as immune-potentiating agents to enhance host protective immunity against enteric pathogens in broilers chickens.

Tissue Distribution of Bovine Spongiform Encephalopathy in Cattle

Bovine spongiform encephalopathy (BSE), a fatal neurodegenerative disease that affects cattle, exotic ungulates, cats, and humans, is a transmissible spongiform encephalopathy (TSE). TSEs are caused by infectious proteins called prions that are resistant to various

methods of decontamination and environmental degradation. BSE in cattle is a feed-borne disease caused by ingesting feedstuffs contaminated with meat and bone meal containing tissue from infected cattle. Some countries have established feed bans to prevent mammal-derived proteins from entering the ruminant food chain. Furthermore, an effort has been made to identify the tissues that contain prions in cattle affected with BSE, called specified risk materials (SRMs) and eliminate them from the human food chain. In the past, SRMs have been identified using techniques with limited sensitivity. ARS scientists in Ames, Iowa, used an ultra-sensitive technique to examine tissues from cattle with clinical signs of BSE. This technique, protein misfolding cyclic amplification (PMCA), amplifies miniscule amounts of material to detectable levels. PMCA was used to demonstrate abnormal prion in a number of tissues that confirm previous results obtained through traditional techniques and validated the use of this PMCA protocol. In addition, positive results were obtained from a number of tissues in which abnormal prion had not previously been detected including esophagus, adrenal gland, rumen, and rectum. These tissues represent a previously unrecognized risk for BSE transmission. These new data demonstrate an expansion in the scientific knowledge that should be considered by regulatory officials when determining guidelines for ruminant feed bans and SRMs to protect animal and human health.

Vitamin D as an Alternative for Antibiotics for Controlling Mastitis

Mastitis is both the most prevalent infectious disease in dairy herds and the most costly disease for dairy producers. Older cost estimates for mastitis are in the neighborhood of $2 billion per year for producers. Antibiotics are the mainstay for mastitis treatment and control. Dairy cattle with mastitis receive more antibiotic therapy for its prevention and treatment than for all other dairy cattle diseases combined. Valid concerns by consumers regarding antibiotic usage need to be addressed by research on non-antibiotic alternatives. A significant proportion of clinical mastitis cases occur in cows in the weeks shortly after calving when the cow's innate immune system is compromised, highlighting the important role of a fully functional immune system in the fight against mastitis. The physiological role of the vitamin D system continues to evolve beyond calcium and skeletal homeostasis to include significant roles in modulating innate and adaptive immune function. It has long been recognized that vitamin D deficiency, as reflected in serum $25(OH)D_3$ concentrations, causes decreased resistance to infection. Recently, vitamin D has been shown to play a role in regulating the ability of immune cells to kill pathogens. There is a lack of $25(OH)D_3$ in the milk compartment of the mammary. In preliminary data, $25(OH)D_3$ was infused into an infected mammary quarter of cows. There was a reduction of mastitis severity with use of vitamin D by impacting the molecular and cellular pathways of immune cells in the mammary gland and may be an important non-antibiotic option for mastitis treatment. Vitamin D is a simple and natural immune stimulator which, in combination with current antibiotics, could become an effective therapy for mastitis. In addition, vitamin D's ability to stimulate the immune system could reduce the time and amount of antibiotics needed to treat mastitis. This combination therapy may cure mastitis infections that are currently resistant to antibiotic treatment alone. The results of reduced antibiotic use would be a reduction in antibiotic residues that may get into the food supply, a reduced potential of antibiotic resistance, and an increase in consumer confidence and international trading opportunities.

Detection of Mycobacterium bovis in Deer

The mainstay of the bovine tuberculosis (TB) eradication program has been the tuberculin skin test, combined with slaughter of both infected and exposed animals (whole herd depopulation), and with indemnity payments to producers paid by both USDA and state authorities. Although this traditional test and slaughter policy has been effective in lowering the prevalence of disease, eradication of bovine TB in the United States has not been achieved in spite of almost 93 years of concerted effort. Additionally, new obstacles have emerged with changes in the livestock industry, trade policies, and the increasing popularity of the captive deer species (cervid) industry. In particular, the current obstacles are: importation of cattle from Mexico infected with tuberculosis; a reservoir of infection in free-ranging white-tailed deer in Michigan; continued detection of TB in captive cervids resulting in transmission to cattle; and persistence of *Mycobacterium bovis* (*M. bovis)* infection in large dairy herds. Farmed deer represent a significant alternative livestock industry, with numbers exceeding 2 million in New Zealand, 1 million in China, 500,000 in the United States, 400,000 in Russia, and 100,000 in Canada. Farmed deer are exposed to various other livestock and to free-ranging wildlife, and are moved between herds and across borders. Thus, there is an increased risk of disease infection among and between farmed deer, traditional livestock, and free-ranging wildlife. Free-ranging and captive deer are implicated in the spread of *M. bovis* to cattle and to humans in the United States, Canada, and New Zealand. The only approved test for use in deer and elk has been the tuberculin skin test. Skin test procedures are problematic in captive cervid species because of the variable accuracy of the test, and injury risks due to handling of the animals (i.e., skin test requires two handling events). Each of these obstacles demonstrates the diversity of issues relating to bovine TB control in the United States. The use of a serologic test for the detection of tuberculosis (TB) in elk and fallow deer naturally infected with *M. bovis* was elucidated. Using samples from a heavily infected captive elk and fallow deer herd (~70% prevalence), we demonstrated that two serum-based tests, which detect TB-specific antibodies, provided much improved accuracy as compared to that achieved with the skin test. In association with prior studies by our group, the collective impact is that a blood-based test is now available for use in captive cervids, pending approval by the U.S. Animal Health Association TB committee and the USDA TB program staff for official use in the eradication program. The proposed research results will have a positive benefit for livestock and captive cervid producers, wildlife agencies, the general public, and USDA action agencies such as USDA/APHIS in controlling the spread of TB in humans and animals.

Brucellosis Control in Wild Animal Reservoirs

Brucellosis is one of the most significant zoonotic diseases of livestock worldwide. *Brucella* species are an important cause of abortion in cattle, small ruminants, and swine. An intensive eradication program for Brucellosis has been ongoing in the United States for more than 50 years. *Brucella* species tend to have a predilection for specific animal species (e.g. *B. abortus*-cattle, *B. suis*-pigs, *B. melitensis*-sheep and goats) but most can infect all mammals with varying degrees of virulence. Wildlife species serve as important reservoirs for *Brucella*; for example, bison and elk in Yellowstone Park infect cattle, and

feral pigs infect both domestic pigs and cattle. The goal of this research project is to increase understanding of the pathophysiology and host-pathogen interaction. From this knowledge, the goal is to develop improved diagnostic assays and vaccines to control and further eradicate the various *Brucella* species. This past year we assessed the ability of current *B. abortus* vaccines to protect cattle against *B. suis.* Additionally, we are assessing the ability of cattle vaccines to protect wildlife against Brucellosis and reduce disease; thus reducing the threat to our domestic animals. This research will assist in developing control strategies for *Brucella* species in both our domestic animals as well as reducing the level of infection in wild animal reservoirs.

Improved Diagnostics for Johne's Diagnostics

Johne's disease (paratuberculosis) is a chronic, progressive enteric disease of domestic and wild ruminants caused by the *Mycobacterium avium* subspecies *paratuberculosis* (MAP). A large percentage of herds in the United States are infected, impacting intrastate, interstate and international movement of cattle. Because of this, it has been estimated to cost the cattle industry more than $1 billion annually. Cattle are infected as calves, but do not show clinical disease until older, typically 2-5 years of age. During this asymptomatic subclinical period, diagnosis is difficult due to the impact of the organism on the immune system. Current vaccines reduce the severity of clinical disease, but do not prevent infection or disease. Improved diagnostics and vaccines are needed to address the problems associated with Johne's disease. Recently, antibodies to novel proteins of MAP have been discovered and used in new diagnostic assays to detect MAP-infected cows using a serological test. This test will be critical in helping to control the disease and elimination of cattle in early stages of infection.