Animal Health (NP 103) Annual Report for 2008

Introduction

The mission of the Animal Health National Program 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 Thacker, National Program Leaders (NPL), for Animal Health, manage the program.

The Animal Health National Program started the new five-year national program cycle Fiscal Year (FY) 2007. The Animal Health National Program currently includes approximately 43 core research projects supported by 118 scientists located at 11 research sites throughout the country. The ARS research budget for the Animal Health Program FY 2008 was $65,356,217.

Critical to the success of the research program is the ability to have access to high containment research facilities and the laboratory equipment necessary to study priority pathogens that pose the highest risks to animal and human health. ARS continues to upgrade its animal research facilities to ensure all research programs are implemented to the highest levels of quality and safety standards. Our new high containment bio-safety level-3 (Ag) large animal facility (Building 9) located at the National Animal Disease Center is now fully operational but delays have been encountered with the final stages of validation. The target date using Building 9 in the implementation of our research programs is Spring 2009.

ARS scientists working in the Animal Health National Program again received several accolades this past year. Darrell R. Kapczynski, Southeast Poultry Research Laboratory, Athens, Georgia, received a Scientist of the Year Award for the development of improved control measures to protect poultry against avian viral diseases. Dr. Hyun Lillehoj, Animal Parasitic Diseases Laboratory, Animal & Natural Resources Institute, was recognized for outstanding research and global technology transfer activities contributing to the development of drug-free control strategies against poultry mucosal pathogens. Dr. Erica Spackman, Microbiologist, Southeast Poultry Research Laboratory, Athens, Georgia, was this year's South Atlantic Area Early Career Research Scientist. Dr. Spackman's citation reads, "Timely development of Rapid Diagnostic Tests for the control of important poultry diseases, including Avian Influenza, Newcastle Disease Virus and Enteric Viruses of Turkeys."

Scientists within the National Animal Health Program were very active in their fields FY 2008, with 289 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 6 articles in trade journals and book chapters. Technology transfer activities for the National Animal Health Program included 26 invention disclosures, 4 new Cooperative Research and Development Agreements (CRADA), 26

active Specific Cooperative Agreements (SCA), and 127 Material Transfer Agreements (MTA).

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

Animal Health Research Highlights

Identification of a New Anti-Infective Protein Secreted by Activated Avian Lymphocytes
Consumers continue to have increasing concerns over the use of antibiotics to control diseases in farm animals. ARS scientists at the Beltsville Animal Parasitic Diseases Laboratory used a high-throughput sequencing strategy to discover a new avian protein in activated intestinal lymphocytes that may have the ability to control coccidiosis. The DNA sequence of the gene coding for this protein is homologous to human NK-lysin. Although the chicken NK-lysin showed relatively low amino acid sequence similarity to mammalian NK-lysins (< 20%), it possessed the characteristically conserved amino acid residues that are the hallmarks of the saposin protein family antimicrobial activity. Although preliminary studies demonstrated an anti-tumor effect, chicken NK-lysin lacked antibacterial activity. Chicken recombinant NK-lysin was cytotoxic for *Eimeria acervulina* and *Eimeria maxima* parasites indicating its important role in innate immune response to avian coccidiosis. Future studies using synthetic peptides derived from NK-lysin may be useful for pharmaceutical and agricultural uses in the food animal industry. This is the first isolation of an anti-infective protein from intestinal lymphocytes.

Scientific Publication:
Hong, Y.H., Lillehoj, H.S., Siragusa, G.R., Bannerman, D.D., Lillehoj, E.P. 2008. Antimicrobial activity of chicken NK-lysin against Eimeria sporozoites. Avian Diseases. 52:302-305.

Identification of H2N3 Influenza A Viruses from Swine in the United States
Although viruses of each of the 16 influenza A HA subtypes are potential human pathogens, only viruses of the H1, H2, and H3 subtype are known to have successfully established infections in humans. H2 influenza viruses have been absent from human circulation since 1968, and as such pose a substantial human pandemic risk. ARS National Animal Disease Center (NADC) scientists reported this year the isolation and characterization of an avian/swine reassortant H2N3 influenza A virus isolated from diseased swine from two farms in the United States. This virus contained an amino acid on the H2 protein that has been associated with increased binding affinity to the mammalian receptor for influenza viruses, and the H2N3 viral isolate was shown to cause disease in experimentally infected swine. In addition, the swine H2N3 virus was infectious and highly transmissible in swine and ferrets. These findings suggest that this H2N3 virus has undergone some adaptation to the mammalian host and that its potential spread should be very closely monitored. Access to the virus for potential vaccine development is available should the H2N3 swine influenza virus re-emerge and begin to circulate among the U.S swine population.

Scientific Publication:
Ma, W., Vincent, A.L., Gramer, M.R., Brockwell, C.B., Lager, K.M., Janke, B.H., Gauger, P.C., Patnayak, D.P., Webby, R.J., Richt, J.A. 2007. Identification of H2N3 influenza A viruses from swine in the United States. Proceedings of the National Academy of Science (PNAS). 104(52):20949-20954.

Domestic Pigs Have Low Susceptibility to H5N1 HPAI Viruses

A H5N1 highly pathogenic avian influenza (HPAI) virus has recently emerged in waterfowl in that is deadly to poultry and humans. Genetic reassortment of H5N1 HPAI viruses with currently circulating human influenza A virus strains could lead to efficient human-to-human transmission and result in an influenza pandemic. Domestic pigs, which are susceptible to infection with both human and avian influenza A viruses, are one of the natural hosts where such reassortment events could occur. ARS scientists at the National Animal Disease Center (NADC) in collaboration with ARS scientists at the Southeast Poultry Research Laboratory (SEPRL) conducted a study in two to three-week-old domestic piglets that were intranasally inoculated with four H5N1 HPAI viruses. Swine H3N2 and H1N1 viruses were also studied as a positive control for swine influenza virus infection. Replication of all four H5N1 viruses in pigs was restricted to the respiratory tract, mainly to the lungs. Titers of H5N1 viruses in the lungs were lower than those of swine viruses. H5N1 viruses were isolated from nasal turbinate of infected pigs. Histological examination revealed mild to moderate bronchiolitis and multifocal alveolitis in the lungs of pigs infected with H5N1 viruses, while infection with swine influenza viruses resulted in severe tracheobronchitis and bronchointerstitial pneumonia. Pigs had low susceptibility to infection with H5N1 HPAI viruses. Inoculation of pigs with H5N1 viruses resulted in asymptomatic to mild symptomatic infection restricted to the respiratory tract and tonsils in contrast to mouse and ferrets animal models, where some of the viruses studied were highly pathogenic and replicated systemically. These results suggest swine have a low susceptibility to these H5N1 viruses and may not play a role in their transmission.

Scientific Publication:
Lipatov, A.S., Kwon, Y., Sarmento, L., Lager, K.M., Spackman, E., Suarez, D.L., Swayne, D.E. 2008. Domestic pigs have low susceptibility to H5N1 highly pathogenic avian influenza viruses. Public Library of Science for Pathogens [serial online]. 4(7):e1000102. Available: http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1000102

Reducing the Dose of Avian Influenza Vaccines is not a Good Idea

Vaccination is an emergency tool that can be used to confront outbreaks of H5N1 high pathogenicity avian influenza (HPAI), but the number of vaccine doses in the U.S National Veterinary Stockpile is limited. To determine if the available vaccine doses could be stretched by using reduced vaccine dose, but maintain adequate efficacy, a vaccination-challenge study was conducted in chickens. At full, half, one-fourth and 1/10 of the avian influenza (AI) vaccine doses, all AI vaccinated chickens were protected from disease and death, but using less vaccine than the full dose had some negative effects including reduced serological titers, more chickens excreting challenge virus, and

higher quantities of challenge virus growth and excretion from intestines and respiratory system. Use of the full vaccine dose is especially important because protection in commercial chickens in the field is typically less than seen in experimental studies in specific pathogen free chickens in the laboratory.

Scientific Publication:
Goetz, S., Spackman, E., Hayhow, C., Swayne, D.E. 2008. Assessment of reduced vaccine dose on efficacy of an inactivated avian influenza vaccine against an H5N1 high pathogenicity avian influenza virus. Journal of Applied Poultry Research. 17:145-150.

Characterization of H5N1 LPAI Avian Influenza Viruses from North America
Avian influenza viruses of many different antigenic subtypes (H1-H16) are found commonly in wild birds, but only the H5 and H7 subtypes are known to have the potential for being highly pathogenic in poultry. The H5N1 subtype is of particular importance because of the widespread outbreaks of highly pathogenic avian influenza in Europe, Asia, and Africa, and extensive surveillance of wild birds was conducted in the Americas to evaluate the chance of these highly pathogenic viruses entering the United States through wild birds. Several H5N1 low pathogenic avian influenza (LPAI) viruses were isolated in wild birds by ARS scientists in collaboration with Animal and Plant Health Inspection Service (APHIS) and United States Geological Survey (USGS), and these viruses were sequenced and shown to be of North American lineage that are separate from the H5N1 highly pathogenic avian influenza (HPAI) viruses found in Europe, Asia, and Africa. The biologic and sequence characterization of these viruses continue to provide evidence that H5N1 HPAI viruses have not traveled to the Americas in wild birds, and clearly documents that H5N1 LPAI viruses are normally found at a low prevalence level in the Americas. This study also included experimental animal studies in collaboration with The Ohio State University that showed that these viruses did not replicate well in poultry and pose only a small threat of introduction to our poultry populations.

Scientific Publication:
Spackman, E., Swayne, D.E., Suarez, D.L., Senne, D.A., Pedersen, J.C., Killian, M.L., Pasick, J., Handel, K., Pillai, S.P., Lee, C., Stallknecht, D., Slemons, R., Ip, H.S., Deliberto, T. 2007. Characterization of lowpathogenicity H5N1 avian influenza viruses from North America. Journal of Virology. 81(21):11612-11619.

Development of Avian Influenza Viruses Rapid Diagnostic Tests
ARS scientists at SEPRL, Athens, Georgia, developed and bench validated a new Real Time RT-PCR protocols for the rapid detection of the subtypes H6, H9 and H11 of avian influenza viruses. Avian influenza virus has 16 distinct antigenic subtypes, but certain subtypes are responsible for most diseases outbreaks in poultry. A real-time reverse transcriptase polymerase chain reaction (RT-PCR) test for the rapid identification of H6, H9, and H11 subtypes of avian influenza were developed and bench validated. The rapid detection and identification of avian influenza viruses, particularly the H6 and H9 subtypes, provide additional tools to diagnose avian influenza outbreaks. The H6 and H9

subtypes are commonly found in other countries and rapid diagnostic tools are needed to rapidly diagnose if these subtypes infect poultry in the United States.

Scientific Publication:
Das, A., Suarez, D.L. 2007. Development and bench validation of real-time reverse transcription polymerase chain reaction protocols for rapid detection of the subtypes H6, H9, and H11 of avian influenza viruses in experimental samples. Journal of Veterinary Diagnostic Investigation. 19:625-634.

Wildlife Present a Potential Source of Bovine Viral Diarrhea Virus (BVDV) for Cattle

Bovine Viral Diarrhea Virus (BVDV) is a costly disease that affects cattle and other ruminants. The virus has many nasty effects, including fever, diarrhea, respiratory and reproductive disease, abortion, birth defects, and death. BVDV is thought by many to be the most important endemic viral disease of cattle, with economic losses estimated at about $50-100 per cow. Design of effective programs geared toward the eradication of BVDV in domestic cattle will require an understanding of BVDV infections in wild ungulates, which are frequently in contact with domestic cattle. ARS scientists at NADC, Ames, Iowa, investigated the potential for does to become persistently infected and serve as a source of infection for our domestic cattle herds. White-tailed does were infected with BVDV, isolated from deer in the field, during the first trimester of pregnancy. Infection resulted in death or reproductive failure (abortion, readsorption, stillbirth) in 11 out of 13 naïve does. Histological and immunohistochemical examination of persistently infected fawns revealed that BVDV antigen was distributed widely throughout many tissues and cell types, most notably epithelium and vascular endothelium, consistent with that reported in cattle. In contrast to cattle, lymphocytes exhibited only very rare positive staining. These findings indicate that BVDV infection results in clinically severe reproductive disease in deer and that there may be differences in the way the virus behaves in deer compared to cattle. These findings suggest that the impact of BVDV reproductive disease in deer may be under appreciated and that because the virus may be spread differently from deer than cattle, different control strategies may be needed.

Scientific Publication:
Ridpath, J.F., Mark, C., Chase, C.L., Ridpath, A.C., Neill, J.D. 2007. Febrile response and decrease in circulating lymphocytes following acute infection of white tail deer fawns with either a BVDV1 or a BVDV2 strain. Journal of Wildlife Diseases. 43(4):653-659.

Development of a New Protective Vaccine for Brucellosis in Bison

Brucellosis is a bacterial disease that can infect domestic cattle and humans. Currently, one of the most common sources of Brucellosis in cattle in the Western States is from contact with infected Bison. Bison appear to be more susceptible to Brucella infection and the clinical effects of disease then cattle. Tools are needed to reduce the high prevalence of Brucellosis in bison in Yellowstone National Park which will help prevent the spread of the organism to cattle in the area. ARS scientists at the National Animal Disease Center (NADC), Ames, IA evaluated the ability of a new recombinant brucellosis vaccine to protect against infection and disease in bison. The new vaccine

was safe for use in bison following experimental challenge, although not as protective against disease and bacterial shedding as observed in vaccinated cattle. This research suggests that while the current vaccines may help reduce brucellosis in bison, better vaccines need to be developed that will more effectively reduce disease and organism shedding in bison.

Documentation and Diagnosis of a Unique Leptospira interogans serovar Pomona in California Sea Lions

Leptospirosis is a bacterial disease that can be spread from infected animals to humans via urine. A new species of Leptospira organisms was recently isolated from sea lions in California. Sea lions infected with Leptospira are a potential public health hazard due to the possibility of spread from infected animals to humans. Infection with Leptospira interogans serovar Pomona causes the sea lions to beach themselves increasing the opportunities for exposure of humans to the bacteria as they interact with the sick sea lions. Currently, the diagnostic tools for detecting Leptospira in sea lions are slow and lack the ability to confirm infection in all animals. ARS scientists at the National Animal Disease Center (NADC), Ames, IA are developing an improved diagnostic assay to detect the presence of Leptospira in infected sea lions. The more rapid and accurate diagnostic assay for Leptospirosis infection in the sea lions will reduce exposure time of humans to the infected animals by facilitating the rapid treatment, isolation and quarantine of infected animals.

Scientific Publication:

Cameron, C.E., Zuerner, R.L., Raverty, S., Colegrove, K.M., Norman, S., Lambourn, D.M., Jeffries, S.J., Gulland, F.M. 2008. Detection of Pathogenic Leptospira Bacteria in Pinniped Populations via PCR and Identification of a Source of Transmission for Zoonotic Leptospirosis in the Marine Environment. Journal of Clinical Microbiology. 46(5):1728-1733.

Identification of Novel Antigens in Johne's Disease

Paratuberculosis (Johne's disease) is a chronic wasting enteric disease of ruminants caused by infection with a bacterial pathogen, Mycobacterium avium subsp. paratuberculosis. Johne's disease results in significant economic losses to the cattle industry due to animal culling, reduced milk production, poor reproductive performance and reduced carcass value. Diagnosis of cattle infected with Johne's is difficult due to the long incubation time between infection and the onset of clinical disease. This past year, ARS scientists at the National Animal Disease Center (NADC), in Ames, IA identified 6 novel proteins that may be candidates for an improved diagnostic test for Johne's disease. The scientists identified the proteins through the use of a newly developed 96-spot protein assay. Studies using the protein assay have determined that some proteins can be detected as early as 70 days after infection of cattle with the M. paratuberculosis. Early diagnosis of infected cattle will allow improved control strategies on a herd basis through isolation and culling of infected animals.

Scientific Publications:

Bannantine, J.P., Waters, W.R., Stabel, J.R., Palmer, M.V., Li, L., Kapur, V., Paustian, M. 2008. Development and Use of a Partial Mycobacterium avium subspecies paratuberculosis Protein Array. Proteomics. 8(3):463-474.

Bannantine, J.P., Paustian, M., Waters, W.R., Stabel, J.R., Palmer, M.V., Li, L., Kapur, V. 2007. Profiling Host Antibody Responses to Mycobacterium avium subspecies paratuberculosis Infection Using Protein Arrays. Infection and Immunity. 76(2):739-749.
Bannantine, J.P., Rosu, V., Zanetti, S., Rocca, S., Ahmed, N., Sechi, L.A. 2008. Antigenic Profiles of Recombinant Proteins from Mycobacterium avium subsp. paratuberculosis in Sheep with Johne's Disease. Veterinary Immunology and Immunopathology. 122(1-2):116-125.

Yakes, B.J., Lipert, R.J., Bannantine, J.P., Porter, M.D. 2008. Impact of Protein Shedding on Detection of Mycobacterium avium subsp. paratuberculosis by a Whole-Cell Immunoassay Incorporating Surface-Enhanced Raman Scattering. Clinical and Vaccine Immunology. 15(2):235-242.

Yakes, B.J., Lipert, R.J., Bannantine, J.P., Porter, M.D. 2008. Detection of Mycobacterium avium subsp. paratuberculosis by a Sonicate Immunoassay Based on Surface-Enhanced Raman Scattering. Clinical and Vaccine Immunology. 15(2):227-234.

Stable Transfection of a Foreign Gene into Babesia bovis

Texas cattle fever, a serious disease of cattle, was eradicated from the United States by controlling the tick vector, Rhipicephalus microplus. These ticks serve as the source of infection in cattle with the causative agent of Texas cattle fever, Babesia bovis. Control of Texas cattle fever has historically been by eliminating the tick. Recent emergence of acaracide resistant ticks has increased the potential for Texas cattle fever to re-emerge in the cattle population in the U.S. New methods to control the ticks and/or the B. bovis organism are needed to control this potentially serious disease of cattle. ARS scientists from the Animal Research Unit in Pullman, WA in collaboration with Washington State University have transfected (inserted) a foreign gene into the red blood cell stage of B. bovis. This ability to alter the genetics of B bovis will enable scientists to insert other genes such as those coding for tick antigens into the organism. The insertion or altered genes in the B. bovis organism will allow the scientists to identify genes and proteins which could be used to induce protective immunity against the ticks in cattle. In addition, this technique will allow scientists to identify the genes used by B. bovis to cause disease (virulence factors) and identify new and novel vaccine candidates. This information is critical for the development of anti-babesial vaccines including those which block transmission between animals.

Scientific Publication:

Suarez, C.E., Mcelwain, T.F. 2008. Transient transfection of purified Babesia bovis merozoites. Experimental Parasitology. 118(4):498-504.