Animal Health (NP 103) Annual Report for 2007

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, DVM, Ph.D, National Program Leader (NPL), Animal Health, is currently managing the program.

The Animal Health National Program started a new five-year national program cycle in Fiscal Year (FY) 2007. The Animal Health National Program currently includes 48 core research projects supported by 124 scientists located at 11 research sites throughout the country. The ARS research budget for the Animal Health Program FY 2007 was $63.5 million (NTL).

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 biosafety level-3 (Ag) large animal facility (Building 9) located at the National Animal Disease Center in Ames, Iowa, is now fully operational and undergoing the final stages of validation.

Several of the scientists in the Animal Health National Program again received accolades this past year. Dr. David Swayne of the Southeast Poultry Research Laboratory in Athens, Georgia, was awarded the Laboratory Director of the Year Award by the Federal Laboratory Consortium for Technology Transfer. The Poultry Science Association awarded Dr. Hyun Lillehoj of the Animal Parasitic Diseases Laboratory in Beltsville, Maryland, the Embrex Fundamental Award in Science.

Scientists within the National Animal Health Program were very active in their fields in FY 2007, with 79 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 22 articles in trade journals and book chapters. Technology transfer activities for the National Animal Health Program included 10 invention disclosures, 7 new Cooperative Research and Development Agreements (CRADA), 35 active Specific Cooperative Agreements (SCA), and 100 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

Understanding the Genetic Basis of Marek's Disease Virus Virulence

Marek's disease (MD) is perhaps the most insidious virus affecting the poultry industry. Significant success in the control of MD has been achieved through the use of vaccines that prevent tumor development (The first cancer vaccine ever developed). However, current vaccines do not block viral infection and spread. It is speculated that vaccine selection pressures have resulted in new highly virulent viral strains that have been reported to cause greater than 50 percent mortality in certain flocks. Continued reports of periodic MD outbreaks in vaccinated flocks all point to the need for new strategies to control this re-emerging viral disease. ARS scientists at the Southeast Poultry Research Laboratory (SEPRL) in Athens, Georgia, and the Avian Disease Oncology Lab (ADOL) in East Lansing, Michigan, completed a comparative genomic project involving 13 virus strains from five MD virus (MDV) pathotypes (very virulent plus, very virulent, virulent, mildly virulent and attenuated). It was discovered that attenuated strains (e.g., strains limited in their disease causing capacity and often used as vaccines) contain altered DNA structure and those specific changes in the DNA likely contribute to their inability to cause disease. The identification and characterization of genetic changes (i.e., polymorphisms) within the viral genome of the 5 pathotypes increases our understanding of virulence, pathogenesis, and will lead to the development of improved vaccines.

Scientific Publication:

Spatz, S.J., Nair, V. 2007. Comparative full-length sequence analysis of oncogenic and vaccine (Rispens) strains of Marek's disease virus. Journal of General Virology. 88:1080-1096.

Spatz, S.J., Silva, R.F. 2007. Sequence determination of variable regions within the genomes of Gallid herpesvirus-2 pathotypes. Archives of Virology. Available: http://www.springerlink.com/content/ku4064776312w861/.

Jarosinski, K.W., Margulis, N.G., Kamil, J.P., Spatz, S.J., Nair, V.K., Osterrieder, N. 2007. Horizontal transmission of Marek's disease virus requires both the unique-long (UL) 13 protein kinase (UL13) and the UL44 glycoprotein C. Journal of Virology. Available: http://jvi.asm.org/cgi/reprint/JVI.01065-07v1?view=long&pmid=17634222.

Designing a Classical Swine Fever (CSF) Vaccine for Control and Eradication

Classical Swine Fever Virus (CSFV) is a highly infectious disease of pigs (related to Bovine Viral Diarrhea Virus (BVDV) in cattle) that results in high rates of mortality and morbidity. Transmission of the virus is horizontal and vertical, resulting in the presence of symptomatic carriers that maintain and spread the virus in an infected herd. CSFV is a significant foreign animal disease threat to the U.S national pork industry. The disease is endemic in areas of Mexico, Central America, and the Caribbean, and thus close to our borders. Recent outbreaks in Western Europe have had devastating economic consequences and have shown that there are critical gaps in the countermeasures available to control this disease. Critical needs are improved diagnostic tests and the discovery of highly effective vaccines that can provide rapid protection and stop the

transmission of the virus, and engineered so that infected pigs can be differentiated from vaccinated pigs. ARS scientists at the Plum Island Animal Disease Center, Orient Point, New York, are conducting molecular virology studies in secure biocontainment facilities to understand the mechanisms of CSF viral pathogenesis, which is a vital step on the path to vaccine discovery. The results of these studies have shown that CSFV contains in its envelope three heavily glycosylated (sugar-coated) proteins (E0, E1 and E2), which play a critical role in virulence and immune response. The role of the glycosylation of these proteins in virulence was established using reverse genetics techniques to alter, delete, and analyze CSFV glycosylation sites. The major accomplishment of this project is the discovery, mapping and characterization of viral genetic determinants of virulence and their manipulation in order to develop new vaccine strains. Specifically, the basic knowledge of the glycosylation patterns of CSFV structural proteins E0 and E2 has been determined along with their role in virus replication *in vitro* (in the test tube) and *in vivo* (in pigs). Manipulation of the E2 glycosylation site has allowed the development of an experimental vaccine strain that has been shown to induce protection as early as 3-days post vaccination. The manipulation of the glycosylation sites also presents opportunities for developing negative marker attenuated vaccines able to induce early protection. (National Program 103 and Performance Measures 3.2.1 and 3.2.3)

Scientific Publication:

Fernandez-Sainz, I., Holinka, L.G., Lu, Z., Risatti, G.R., Borca, M.V. 2007. Removal of a N-linked Glycosylation Site on the Classical Swine Fever Virus Strain Brescia E(rns) Glycoprotein Affects Virulence in Swine. Journal of Virology 81(2): 924-33

Understanding Avian Influenza and the Host Immune Response

The threat of an avian influenza outbreak is significant. Highly pathogenic avian influenza (HPAI) can result in as much as 90 percent mortality in infected flocks. HPAI viruses impact international trade by inhibiting exports from an infected country. An outbreak of AI in the United States would devastate our poultry industry and curtail the availability of poultry meat. HPAI is also a potential zoonotic agent. The continued reports of H5N1 infections in humans in Asia has the public health community concerned that a worldwide pandemic is impending. The most effective way of controlling many zoonotic diseases is at the source (the so called host reservoir). In the case of AI, migrating wild birds harbor the virus and this disease is therefore not eradicable. However, the source of infection for people is most likely to be our domestic poultry. Therefore, expending resources to control and eradicate an outbreak at this level is the best strategy for safeguarding public health and preventing a potential pandemic. ARS scientists at the Southeast Poultry Research Laboratory in Athens, Georgia, have been conducting studies in chickens to understand the mechanisms the virus uses to escape the immune protective responses of the host. Understanding the immune response of chickens to avian influenza virus is important to help us better target vaccines for control of the disease. The interferon response in chickens plays an important role in the control of avian influenza virus. Two variants of influenza, one that had a full length NS1 protein and the other with only a partial length NS1 protein, were compared and it was demonstrated that the virus with a short NS1 protein induced higher levels of interferon

that resulted in poor growth of the virus in chickens. The NS1 protein had previously been shown to be an important interferon blocker in mammals, but this study showed it has the same role in chickens and has expanded our understanding of the pathogenesis of the virus. (National Program 103 and Performance Measure 3.2.1).

Scientific Publication:

Cauthen, A.N., Swayne, D.E., Sekellick, M.J., Marcus, P.I., Suarez, D.L. 2007. Amelioration of influenza pathogenesis in chickens attributed to the enhanced interferon-inducing capacity of a virus with a truncated NS1 gene. Journal of Virology. 81(4):1838-1847.

The Risk of Low Pathogenic Avian Influenza Viral Strains Becoming Virulent

The use of reverse genetics to make avian influenza viruses with a specific sequence has proven to be a valuable tool to examine how these viruses cause disease in poultry. From 1994 to 2006 low pathogenic H7N2 avian influenza viruses circulated in the live bird markets of the Northeast United States, and there has been considerable concern that these viruses might change or mutate to the virulent form of the virus. Using reverse genetics techniques, ARS scientists at the Southeast Poultry Research Laboratory in Athens, Georgia, have taken a representative H7N2 virus and genetically changed the virus to try and understand the minimum number of nucleotide changes needed for the virus to become virulent. The results of this study showed that the virus needed insertions of amino acids at a key site in the virus, the cleavage site, to become virulent, and simple mutations at the cleavage site by themselves would not make the virus virulent. This study improved our understanding of how avian influenza viruses become virulent and will help us understand risks of low pathogenic viruses changing to the highly pathogenic form in the future. (National Program 103 and Performance Measure 3.2.1).

Scientific Publication:

Lee, C., Lee, Y., Senne, D.A., Suarez, D.L. 2006. Pathogenic potential of North American H7N2 avian influenza virus: A mutagenesis study using reverse genetics. Virology. 353:388-395.

Wood Ducks are Highly Sensitive to Avian Influenza

Since 2002, H5N1 HPAI viruses have caused mortality in numerous species of wild aquatic birds in Asia and Europe. Some HPAI viruses cause severe disease in several species of wild ducks in experimental infections. In collaboration with the Southeastern Cooperative Wildlife Disease Center (University of Georgia), ARS scientists at the Southeast Poultry Research Laboratory (Athens, Georgia) evaluated five species of wild ducks intranasally inoculated with an Asian strain of H5N1 HPAI virus. The wood duck was 2-4 times more susceptible to infection than chickens, the latter being highly susceptible to the virus. Mallards (Anas platyrhynchos), northern pintails (Anas acuta), blue-wing teals (Anas crecca), and redheads (Aythya americana) were less sensitive to infection, produced virus in low concentrations for short periods of time, and did not

exhibit clinical signs. These data suggest that the wood duck would represent a sensitive indicator species for H5N1 HPAI should it entered North America. (National Program 103 and Performance Measure 3.2.1).

Scientific Publication:

Brown, J.D., Stallknecht, D.E., Beck, J.R., Suarez, D.L., Swayne, D.E. 2006. Susceptibility of North American ducks and gulls to H5N1 highly pathogenic avian influenza viruses. Emerging Infectious Diseases. 12(11):1663-1670.

Comparison of the Innate Immune Response to Avian Influenza in Chickens and Ducks

Wild birds, especially ducks and shorebirds, are reservoirs of Avian Influenza (AI) viruses. Excrement and respiratory fluids from infected wild birds are the most important source of Avian Influenza virus. Virus shedding can be detected as early as 1 day post infection in poultry and may continue for up to 4 weeks in a population of birds. Comparison of the innate immune response in chickens and ducks to H5N1 avian influenza show a markedly different response between species. The innate immune response is responsible for detecting invading microorganisms during the initial stages of infection, which is a crucial determinant of disease resistance or susceptibility. ARS scientists at the Southeast Poultry Research Laboratory in Athens, Georgia, have been conducting studies to examine the role of the innate immune response in protection from disease by measuring cytokine (factors that modulate the immune response) expression immediately following infection. The results indicate differences in cytokine expression between chickens and ducks following exposure with H5N1 viruses isolates recovered from Southeast Asia. Ducks generally displayed increased cytokine expression and resistance to challenge, while chickens exhibit decreased cytokine expression. These studies emphasize the importance of innate immunity in birds and correlate increased pathogenicity of recent H5N1 viruses for wild waterfowl with an enhanced suppression of the host immune response. (National Program 103 and Performance Measure 3.2.1).

Scientific Publication:

Pantin Jackwood, M.J., Swayne, D.E. 2007. Pathobiology of Asian highly pathogenic avian influenza H5N1 virus infection in ducks. Avian Diseases. 51:250-259.

Cooking Poultry Meat Inactivates Avian Influenza Virus

HPAI viruses can be present in the meat of infected poultry and poses a potential health risk. Research at the Southeast Poultry Research Laboratory in Athens, Georgia, previously showed that cooking was effective in killing an H5N1 HPAI virus. Two additional HPAI viruses (H5N2 Pennsylvania/83 and H5N2 Texas/04) were tested for thermal inactivation in naturally or artificially infected meat. Cooking at 70^{o}C or 73.9^{o}C (165^{o}F) were effective at killing the viruses in less than 1 minute. This study further demonstrates that proper cooking of poultry using the USDA Food Safety Inspection Service (FSIS) salmonella standards would be effective at killing HPAI viruses. (National Program 103 and Performance Measure 3.2.3).

Scientific Publication:

Thomas, C., Swayne, D.E. 2007. Thermal inactivation of H5N1 high pathogenicity avian influenza virus in naturally infected chicken meat. Journal of Food Protection. 70(3):674-680.

Bacteriophage as an Alternative to Anitbiotics for Treating Avian Bacterial Infections
The emergence of bacteria resistant to antibiotics poses a significant threat to animal and human health. There is growing concern that the use of antibiotics in animal production can result in the emergence of resistant bacteria that cause human infections that are difficult to treat. The significance of the use of antibiotics in animal production versus their use in human medicine to the emergence of resistance bacteria that pose a threat to human health has been debated for a long time and is equivocal. With the continued concern over the use of antibiotics in poultry production, there is a real need to find safe and practical alternatives in poultry production to both prevent and treat poultry diseases. ARS scientists at the Poultry Production and Product Safety Research Unit, Fayetteville, Arkansas, are evaluating the efficacy of bacteriophage to be used as an alternative to antibiotics to prevent and treat bacterial respiratory diseases of poultry. Bacteriophage are viruses that kill bacteria without any known activity to animal or plant cells. One of the attributes of bacteriophage is the ability to deliver bacteriophage to the site of infection, whereas delivering therapeutic levels of antibiotics to the lungs is difficult to achieve. Accordingly, bacteriophage therapy has potential as a safe and environmentally benign alternative to antibiotics. One of the concerns with using bacteriophage to treat bacterial infections in animals and humans is that the animal could respond to the bacteriophage treatment by producing antibodies to the bacteriophage, which would limit the efficacy of repeated treatment of chronic bacterial infections. ARS scientists have determined that, indeed, poultry mount an immunological response to bacteriophage that reduces bacteriophage efficacy by approximately 50 percent. However, ARS scientists have developed an *in vitro* assay that can be used to determine if bacteriophage differ antigenically, which would allow the pharmaceutical industry the basis for designing bacteriophage products to prevent immune interference with bacteriophage efficacy. This assay could be used in both animal and human clinical laboratories to customize bacteriophage treatment of bacterial infections. (National Program 103 and Performance Measure 3.2.3).

Scientific Publication:

Huff, W.E., Huff, G.R., Rath, N.C., Donoghue, A.M. 2006. Evaluation of the influence of bacteriophage titer on the treatment of colibacillosis in broiler chickens. Poultry Science. 85:1373-1377.

Epidemiology and Evolution of Exotic Newcastle Disease
Newcastle disease (ND) is an infection of birds caused by a virulent Newcastle disease virus (vNDV) and is a world wide problem for poultry production. The virulent form of the disease, called exotic ND (END), has been eradicated from U.S. poultry, but the threat of introduction of END virus (vNDV), as occurred in 2002 is always present.

Avian paramyxovirus type 1 (APMV-1), synonymous with Newcastle disease virus (NDV), includes a diverse group of viruses that may infect any bird species and the consequence of that infection can range from high morbidity and mortality to little or no clinical disease. Only the infection and disease attributable to vNDV is reportable to the World Organization for Animal Health (OIE). Recent analysis of genomic sequences has revealed the existence of two distinct clades: class I and II. Class I isolates have been primarily recovered from waterfowl and are generally attenuated in poultry. The class II NDV comprise the vast majority of vNDV and include isolates recovered from poultry (gallinaceous birds), pet, and wild bird viruses. The class II NDV are further categorized into genotypes I to IX. While vNDV are not currently found in U.S. poultry, virulent class II viruses are often found in cormorants (genotype V), pigeons (genotype VI), and in poultry in endemic regions of Mexico and Central America (genotype V). The NDV outbreak in California during 2002-2003 was caused by a vNDV of genotype V, which was closely related to viruses isolated from recent outbreaks in Mexico and Central America. Virulent viruses of genotypes VI to IX are often isolated from poultry in many Asian countries, including our larger trading partners, such as China, Taiwan and Korea as well as Iran, and countries of the African continent. The epidemiology and evolution of NDV is not fully understood, and few natural reservoirs have been identified. Previous studies have focused on tracing either the origin of specific vNDV isolates or in characterizing the spread of vNDV in poultry outbreaks, but recent research is starting to shed light on the distribution and epidemiology of NDV. vNDV isolates recovered from pigeons and migrating cormorants have been identified as the likely source of past vNDV outbreaks in poultry. Viruses with potential to cause disease in poultry continue to circulate in feral birds in the U.S., and in poultry and other birds in parts of Mexico and South America. Characterization of NDV infecting waterfowl and shorebirds during 1986-2005 have provided evidence for the existence of natural reservoirs with epidemiological links to poultry in the U.S. and in the Hong Kong live bird markets. The close phylogenetic relationship among some shorebird viruses and live bird market-origin NDV, together with weaknesses in the USDA matrix gene real time (r) reverse transcriptase polymerase chain reaction (RT-PCR) assay to detect class I viruses from these viruses, suggests that viral transmission may occur unnoticed among wild birds and poultry. While it is impossible to predict which genotypes represent the most significant threat to the U.S. poultry industry, the geographic proximity of viruses of genotype V and the large volume of commerce and travel with Mexico and countries of Southeast Asia, suggest that further evaluation of the efficacy of current U.S. vaccines and diagnostic assays on emerging viruses of genotypes V to IX are needed. Widely used B1 and La Sota vaccines and the current challenge test virus (Texas GB) are genotype II viruses that originated in 1947 and differ considerably from emerging vNDV. In addition, there is evidence of the existence of a vast unexplored diversity of NDV that is not captured by the current rapid rRT-PCR tests. ARS scientists at the Southeast Poultry Research Laboratory, Athens, Georgia, have sequenced the fusion protein cleavage site of 21 NDV isolates from the Hong Kong live bird markets, conducted phylogenetic characterization, and developed an alternative rRT-PCR test, which complements the U.S. matrix gene rRT-PCR test. Phylogenetic analysis and preliminary rRT-PCR tests suggest that the newly developed assay can detect a majority of class I isolates from the U.S. These tests will permit the prediction of the virulence potential for Class I viruses isolated in the U.S.

Identifying the genotypes of viruses with potential to acquire virulence upon replication in poultry is an important step in improving our capacity to prevent future outbreaks. (National Program 103 and Performance Measure 3.2.1).

Scientific Publications:

Kim, L.M., King, D.J., Suarez, D.L., Wong, C.W., Afonso, C.L. 2007. Characterization of Class I Newcastle disease virus isolates from Hong Kong bird markets and detection using real-time reverse transcription PCR. Journal of Clinical Microbiology. 45:1310-1314.

Kim, L.M., Afonso, C.L., Suarez, D.L. 2006. Effect of probe-site mismatches on detection of virulent Newcastle disease viruses using a fusion-gene real-time reverse transcription polymerase chain reaction test. Journal of Veterinary Diagnostic Investigation. 18:519-528.

Characterization of Atypical BSE Cases in the United States

Three cases of bovine spongiform encephalopathy (BSE) have been diagnosed in the United States. The first case was found in December 2003 in a cow imported from Canada, and two subsequent cases in cows born and raised in the U.S. The first U.S. indigenous case was found in a downer cow in Texas in November 2004. The second indigenous cow was found on a farm in Alabama in March 2006. The 2003 imported case is reported to have cost the U.S. beef industry $3.2-4.7 billion. ARS scientists at the National Animal Disease Center (NADC) in Ames, Iowa, have now reported that the two indigenous U.S. BSE cases were "atypical." Using a variety of laboratory diagnostic methods to conduct the analyses, NADC scientists did not detect any definite spongiform lesions in the BSE positive brains of the 2004 and 2006 U.S. cases, and saw smaller quantities of the abnormal prion agent, when compared to typical BSE cases. Also, the abnormal forms of the BSE prions in the 2004 and 2006 animals were of a larger size than in typical BSE cases, and the prions reacted differently to a panel of antibodies used to diagnose transmissible spongiform encephalopathies (TSE). To date, only a few atypical BSE cases have been reported in cattle from France, Germany, Japan, the Netherlands, and the United Kingdom. Atypical cases of BSE were a somewhat unexpected finding since it was previously believed that BSE disease in cattle was caused by a single strain of the abnormal prion agent. There are several hypotheses proposed to explain this finding: one theory proposes there are variants of the BSE agent with different molecular features in cattle; a second theory proposes cattle may have been infected by another source of an infectious prion agent (e.g. scrapie or CWD); a third theory proposes a rare sporadic or genetic form of TSE disease could exist in cattle as described for human TSE. There is a paucity of peer reviewed published data on atypical BSE at this time. Important questions will be answered by ongoing and planned studies to determine the tissue distribution of abnormal prions in atypical cases. At this time we know that 1) cases have occurred in different breeds of cattle with different genotypes, 2) the majority of cases occur in older cattle, and 3) we also know that very few of the animals have typical clinical symptoms of BSE. Further research is needed to determine the frequency of these atypical cases, their pathogenesis in cattle and if possible, the

origin of such novel BSE phenotypes. (National Program 103 and Performance Measure 3.2.1)

Scientific Publication:

Richt, J.A., Kunkle, R.A., Alt, D., Nicholson, E.M., Hamir, A.N., Czub, S., Kluge, J., Davis, A.J., Hall, S.M. 2007. Identification and characterization of two bovine spongiform encephalopathy cases diagnosed in the United States. Journal of Veterinary Diagnostic Investigation. 19(2):142-154.

Understanding the Pathogenesis of Johne's Disease

Paratuberculosis (Johne's disease) is a chronic wasting enteric disease of ruminants caused by infection with a bacterial pathogen designated as Mycobacterium avium subsp. paratuberculosis. Economic losses are estimated to be $200/infected cow/year and are the result of animal culling, reduced milk production, poor reproductive performance, and reduced carcass value. Prevalence of Johne's disease in the U.S. dairy cattle population is estimated to be 22-40 percent. This past year, ARS scientists at the National Animal Disease Center (NADC) in Ames, Iowa, have produced several novel monoclonal antibodies (highly specific antibodies engineered to bind to a very precise mark or "epitope" on an antigen) against Mycobacterium avium subsp. paratuberculosis. These antibodies have enabled scientists to follow disease progression as this bacterial pathogen infects its host and have opened up new areas of research in pathogenesis and diagnostic studies. Importantly, ARS have also identified antibodies produced in sheep infected with Johne's Disease that react to both a sheep protein and a protein produced by Mycobacterium avium subsp. paratuberculosis. This finding suggests that the host immune response may not be able to distinguish between self proteins and this bacterial pathogen. This new information provides the first clues to the immunopathology observed in Johne's Disease, and may suggest an autoimmune component to this disease. (National Program 103 and Performance Measure 3.2.1)

Scientific Publications:

Bannantine, J.P., Radosevich, T.J., Stabel, J.R., Sreevatsan, S., Kapur, V., Paustian, M. 2007. Development and Characterization of Monoclonal Antibodies and Aptamers Against Major Antigens of Mycobacterium avium subsp. paratuberculosis. Clinical and Vaccine Immunology. 14(5):518-526.

Bannantine, J.P., Radosevich, T.J., Stabel, J.R., Berger, S., Griffin, J.F., Paustian, M. 2007. Production and Characterization of Monoclonal Antibodies Against a Major Membrane Protein of Mycobacterium avium subsp. paratuberculosis. Clinical and Vaccine Immunology. 14(3):312-317.