National Program Assessment
Animal Health
2000-2004

National Program Assessments are conducted every five-years through the organization of one or more workshop. Workshops allow the Agricultural Research Service (ARS) to periodically update the vision and rationale of each National Program and assess the relevancy, effectiveness, and responsiveness of ARS research. The National Program Staff (NPS) at ARS organizes National Program Workshops to facilitate the review and simultaneously provide an opportunity for customers, stakeholders, and partners to assess the progress made through the National Program and provide input for future modifications to the National Program or the National Program's research agenda. A workshop for Animal Health has been scheduled for October 2004, Greensboro, North Carolina. This report provides the accomplishments for the 2000-2004 National Program Cycle. You will find detailed information on research objectives, achievements, and impact. Results are provided according to location. Comments and assessments should be addressed to mailto:cgg@ars.usda.gov prior to October 2004.

EXECUTIVE SUMMARY

This report provides the accomplishments of research conducted under the Agricultural Research Services (ARS) Animal Health National Program from 2000 to 2004. The purpose of this report is to assess the impact of the program. An assessment is conducted at the end of each 5-year program cycle to update the vision and rationale of the program. The goal of this assessment is to determine whether the program has had an impact on the field of science, animal health, American agriculture, and the U.S. economy.

A summary of the Action Plan prepared at the start of the animal health national program cycle is provided. The Action Plan identified seven research components that are the focus of the Animal Health Research Program: Pathogen Detection and Diagnostics; Microbial Genomics; Animal Immunology; Mechanisms of Disease; Genetic Resistance to Disease; Epidemiology of Disease; and Strategies to Control Infectious and Non-Infectious Diseases. The Action Plan provides the research direction, goals, and the anticipated outcomes and impact. Research plans with specific objectives wre derived from the Action Plan. A total of 60 research projects were identified, subjected to external peer review, and implemented by 18 Research Units located throughout the United States.

Research accomplishments with the greatest impact are summarized for each of the seven research components. The accomplishments are presented relative to their impact on specific diseases. Many diseases affect a single species or are specific to a particular animal industry or management system (e.g., dairy verus beef) but many cross host barriers and affect more than one animal segment (e.g., brucellosis). Thus, the impact of accomplishments can only be assessed through a comprehensive understanding of veterinary medicine, animal health, and the management systems of the applicable animal industries. Because of the tremendous number of variables associated with animal health and various management systems, this report only attempts to assess impact within specific disease areas. The broader impact on specific commodity groups, regulatory and action agencies, American agriculture, the U.S economy, or the field of sciencewill be determined by our customers, stakeholders, and partners. As such, this report will be broadly distributed and subject to an external panel review.

Many of the anticipated outcomes and impacts identified at the start of the animal health national program cycle have been achieved. The research component with the greatest success and impact has been in the area of diagnostic discovery and development. Several new and improved tests have been discovered by ARS scientists. Many of these tests have been commercialized or adopted by animal health officials in the control and eradication of diseases. Many of the diagnostic discoveries have been for zoonotic diseases that impact public health and biological threat agents that could cause tremendous harm to our economy and the Nation.

Animal Health program impact has been achieved in all areas of the action plan;

- The goal of research component #1 “Pathogen detection and diagnositics” was to produce a new generation of diagnostics tools. Diagnostic tools were produced for detection of Anaplasmosis, avian influenza, classical swine fever, chronic wasting disease, Johne’s disease, Listeria moncytogenese, Newcastle disease, Poult enteritis mortality syndrome, toxoplasmosis, trichellosis and tuberculosis.

- The goal of research component #2 “Microbial Genomics” was to use functional genomic analysis of pathogenic microorganisms to solve significant animal disease problems. Microbial genomics was used to understand how avian influenza evolves and adaptes to poultry populations; used to study the differences in low and high virulence BVD viruses in host cells; used to identify specific regions of Johne’s disease and Leptospirosis that can be used as vaccine candidates; used to do a comparative assessment of six different pox viruses which will lead to a better understanding of pox viruses pathogensis.

- The goal of research component #3 “Animal Immunology” was to understand the early development of the immune system allowing scientists to apply new approaches to vaccine delivery and use bio-therapeutics for disase treatment. In this area ARS scientists created reagents for the assessment of immune responses in poultry, “designer” immune reagents to assess swine immune responses and immunomodulators that influence coccidiosis in chickens and bovine mastitis.

- The goal of research component #4 “Mechanisms of Disease” was to understand at the molecular level how pathogens cause disease. The disease mechanisms that were further understood were those of Marek’s disease (genes involved in pathogenicity, replication and cell transformation were identified), pine needle abortion (toxins, toxin producers and toxin producing conditions were identified) and Porcine Reproduction and Respiratory Syndrome (host cell gene expression patterns were determined).

- The goal of research component #5 “Genetic resistance to disease” was to develop means by which the genomes of agricultural species can be utilized to reduce the economic impact of animal diseases. ARS scientists developed a genetic test that can determine if a chicken is resistant to Avian Leukosis virus infection; developed a genotyping system in cattle that can associate alleles with infection phenotype in commercial beef populations; identified several candidate genes in chickens that may confere resistance to Marek’s disease; determined a means by which genetics can be used to breed increased resistance in sheep to Scrapie infection.

- The goal of research component #6 “Epidemiology of Disease” was to incorporate epidemiological analysis into research programs. Molecular epidemiology was used to show that Avian Influenza has circulated in live bird markets for years; that wild birds could be a source of Avian Pneumovirus for turkeys; allow epidemiological tracebacks of Brucellosis; establish the shedding patterns of Malignant Catarrhal Fever virus in sheep; that Neosporosis is a primary cause of abortion in cattle and that dogs are an important on-farm risk factor; white-tailed deer were the primary source of Tuberculsis infection to cattle; Vesicular Stomatitis infections in the USA often originate from Mexico.

- The goal of research component #7 “Strategies to Control Infectious and Non-Infectious Diseases” was to develop and integrate basic research information into applied solution that can be used by farmers and ranchers to control animal disease. In this area ARS scientists have determined a means by which Foot and Mouth Disease virus infections can be stopped; developed a nutrional solution that will help to prevent cows from getting milk fever; developed a method to rapidy and evenly vaccinate laying hens against Mycoplasma infection; developed a candidate vaccine against Newcastle disease that can easily be differentiated from natural infection; a swine influenza vaccine that can be used in the face of maternal antibody.

The contributions made to the field of science by ARS scientists cannot be underestimated and the impact can easily be assessed by reviewing the list of publications provided in the appendix of this report.

BACKGROUND INFORMATION

Agricultural Research Service (ARS)
Role
Mission
National Program Cycle
National Program Assessment
Animal Health National Program (NP 103)

Agricultural Research Service

The Agricultural Research Service (ARS) is the principal in-house research agency of the U.S. Department of Agriculture (USDA). ARS is one of four agencies in the Research, Education, and Economics (REE) mission area. ARS is charged with extending the Nation's scientific knowledge with research projects in agriculture, human nutrition, food safety, natural resources, the environment, and other topics affecting the American people on a daily basis. ARS supports more than 2,100 scientist years organized into approximately 1,050 permanent research projects at over 100 locations across the country and five overseas laboratories and more than 150 librarians, technical information specialists, and other library specialists.

Role

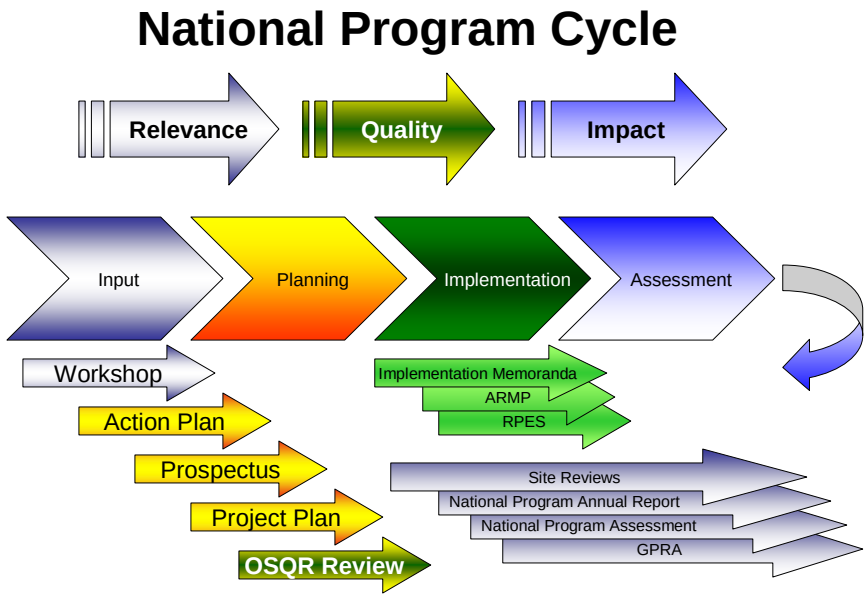
ARS conducts innovative research to find solutions to problems of high National priority. ARS often selects high-risk scientific endeavors to make significant breakthroughs in important problem areas but also complements the work of State Colleges and Universities, State Agricultural Experiment Stations (SAES), other Federal agencies, and the private sector. Mechanisms for addressing State and local issues are already in place; therefore, activities within ARS focus on issues having a regional or national scope and where there is a clear Federal role. ARS also provides research support to USDA action and regulatory agencies and to several other Federal regulatory agencies, including the Food and Drug Administration and the Environmental Protection Agency.

Mission

Our mission is to conduct research to develop and transfer solutions to agricultural problems of high national priority and provide information access and dissemination to:

- Ensure high-quality, safe food, and other products
- Access the nutritional needs of Americans
- Sustain a competitive agricultural economy
- Enhance the natural resource base and the environment
- Provide economic opportunities for rural citizens, communities, and society as a whole

National Program Cycle
The management and execution of all ARS research programs is organized around the five-year National Program Cycle, consisting of four sequential phases designed to ensure the relevance, quality, and impact of every National Program: 1) Input; 2) Planning; 3) Implementation; and 4) Assessment.



National Program Assessment
A National Program Assessment is conducted every five-years through the organization of one or more workshop. Workshops allow ARS to periodically update the vision and rationale of each National Program and assess the relevancy, effectiveness, and responsiveness of ARS research. The National Program Staff organizes National Program Workshops to facilitate the review and simultaneously provide an opportunity for customers, stakeholders, and partners to assess the progress made through the National Program and provide input for future modifications to the National Program or the National Program's research agenda.

Animal Health National Program (NP 103)
The mission of the Animal Health 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 of livestock and poultry. Dr. Robert A. Heckert, National Program Leader, Animal Health, and Dr. Cyril G. Gay, National Program Leader, Animal Health and Safety lead this ARS research program. Dr. Gay is lead on domestic disease issues and vaccine and drug discovery projects, and Dr. Heckert is lead on projects involving foreign and emerging diseases. The Animal Health National Program currently includes 60 research projects supported by 118 scientists located at 14 research sites throughout the country. The ARS research budget for the Animal Health Program is approximately $54.3 million (NET).

ACTION PLAN

Introduction
A workshop for the Animal Health Program was held September 21-23, 1999 in College Park, Maryland. The workshop was attended by 127 stakeholders and 50 ARS scientists. Invited stakeholders were encouraged to send letters presenting their animal health priorities. Input received from our stakeholders was used to formulate an Action Plan for the Animal Health National Program, consisting of seven research components and the anticipated results to be generated from the proposed research. This process was meant to provide a coordinated multi-location plan for research projects conducted by ARS scientists and cooperators that address regional and national priorities and result in fundamental advances in agricultural science. All projects were prospectively reviewed by an external panel in 2001, in accordance with Office of Scientific and Quality Review (OSQR) policy and procedures.

The following outlines the goals of the action plan for each of the seven research components, including the anticipated outcomes and impact identified at the onset of the Animal Health National Program Cycle. The complete action plan can be reviewed on-line at
http://www.ars.usda.gov/research/programs/programs.htm?np_code=103&docid=288

Research Components
Research Component 1: Pathogen Detection and Diagnostics

Goals
To produce a new generation of diagnostic tools are needed that will facilitate detection and identification of known pathogens and diseases, new variants of infectious and non-infectious agents, and emerging organisms and diseases. As appropriate, the procedures will be adapted for use in either field or laboratory diagnostic situations. These new technologies will be transferred to private organizations for the purpose of commercialization, or to state and federal diagnostic laboratories for regulatory or limited field use. Techniques that are applicable to national animal disease programs or human health problems will be standardized and validated through collaboration with other federal agencies to provide a scientific basis for their use in surveillance of animals, animal feeds, animal products, and the environment. Application of these techniques will enhance the general health of U.S. livestock, fish, poultry, and wildlife, and provide assurance that animals and animal products intended for domestic or international trade are free of infectious and toxic agents.

Outcomes
- Availability of more sensitive and specific techniques for diagnosis of animal disease.
- Capability for more rapid identification of new or re-emerging diseases.
- Development of predictive data bases for risk assessment studies.
- Increased domestic and international trade of animals and animal products.
- Reduced public health risk from food-borne illness.
- A safer environment.

Impact
Efficient and profitable animal production systems in which animal well-being is maximized and in which disease detection is rapid and causes minimal impact on production, trade, human health and the environment.

Research Component 2: Microbial Genomics

Goals
To use functional genomic analysis of pathogenic microorganisms, including bacteria, viruses, protozoa, parasites and fungi to solve significant animal disease problems. Specifically to: 1) understand fundamental genetic and biological processes underlying microbial pathogenesis and host resistance; 2) facilitate pathogen gene discovery for use as disease control targets; 3) provide pathogen gene sequences for development of improved gene and protein-based diagnostic tests; and 4) identify pathogen gene targets for development of improved vaccines and therapeutics.

Outcomes
- Improved pathogen detection and diagnosis
- Improved tools for epidemiological disease investigations
- Understanding of new metabolic pathways.
- Improved vaccines and therapeutics
- Novel disease control strategies

Impact
Better and faster means to develop diagnostic tests and track microbial pathogens in animal hosts and the environment, better vaccines and therapeutics and novel disease control strategies based on a new understanding of the molecular mechanism of disease.

Research Component 3: Animal Immunology

Goals
Understanding the early development of the immune system in each of our food animals will enable scientists to apply new approaches to vaccine delivery and to use novel bio-therapeutics for disease treatments. Adapting existing knowledge of rodent and human immunology to domestic animals will speed up the process and cost effectiveness of research. Fundamental research is urgently needed in mucosal immunology and the mechanisms involved in innate and acquired immunity in order to have a solid foundation to improved methods of targeting host/pathogen interactions. Understanding how a host responds to invasion by a pathogen will lead to development of a whole new series of methods to prevent primary infection and abrogate the development of disease.

Outcomes

- New vaccines, better vaccine delivery, new therapeutic interventions and immunomodulators to control or prevent disease.
- New tools to quantitate a broader range of host immune responses for a better understanding of immune function and how to maximize new approaches to vaccine delivery.
- New methods of disease prevention and control.
- Novel vaccines and therapeutics, improved food safety, decreased antimicrobial and pesticide dependence.
- Better immuno-diagnostics and improved nutrition to promote disease resistance.

Impact

New knowledge in animal immunology will enable more rapid progress toward understanding the function of immunity in host/pathogen interactions.

Research Component 4: Mechanisms of Disease

Goals

Understand how pathogens function at a molecular level, how they invade tissue surfaces, how they evade host immune responses and how they interact with higher organisms at a molecular level to cause disease and associated lesions.

Determine which genes encode virulence factors for important pathogens. Identify management practices that can exacerbate virulence mechanisms, e.g., antibiotic usage or specific dietary components. Alter host and pathogen genes to cause and evaluate predicted changes in pathogen virulence as well as latency to prove the relevance of the postulated interactions.

Define how nutrition and basic physiology and metabolism interface with both infectious and noninfectious disease. Define the toxic products of plants and determine how they cause disease in livestock.

Define which pathogens, and specifically which isolate and which genes in the pathogen, are primary factors in disease pathogenesis. In mixed infections determine how each pathogen influences disease pathogenesis. Define strategies to ameliorate the results of complex pathogen interactions. Understand the complexities of the real world interactions of pathogens with each other and with hosts.

Outcomes

- New understanding of complex host/pathogen interactions will lead directly to novel preventative and therapeutic interventions.
- New diagnostic tools and potential intervention strategies that can be tested empirically.
- New strategies for inhibiting pathogen interactions with hosts.
- Generation of effective and safe new drugs, vaccines, and perhaps other products that allow industry to package modern technology into simple solutions for formerly intractable problems.

Impact

Understanding mechanisms of disease will enable more rapid progress toward understanding virulence and host immunity that influence host/pathogen interactions.

New knowledge in this area will enable more rapid progress toward understanding virulence of pathogens and complex interactions between toxins, physiologic and nutritional interfaces with the many environmental and management factors that influence host/pathogen interactions and precipitate disease.

Research Component 5: Genetic Resistance to Disease

Goals

The overall goal of studies into genetic resistance to disease are to develop means by which the vast diversity present in the genomes of important agricultural species can be utilized in integrated systems to reduce the economic impact of important animal diseases. The first step in such a program will be to acquire comprehensive data that adequately defines the genotypic and phenotypic compositions of selected test populations and of lines and breeds used in production systems. Present limitations include inadequate markers for desirable traits, inexact means to precisely measure desirable traits and inadequate tools to delineate associations between genetic structure and the desired traits. As markers for desirable traits and accurate means to measure the traits are perfected it will then become possible to identify genes or genomic regions within genes that are associated with resistance to important diseases of agricultural and public health interest. In addition to collection of genomic data and definition of important phenotypes, it will be necessary to develop adequate bioinformatics systems to facilitate analyses of the vast number of observations expected when measuring complex biologic traits such as disease resistance. As genomic regions are identified as being linked to desirable traits, research efforts will focus on the validation in production animals raised in standard production.

Outcomes

- Identification of genes important in disease resistance.
- Mapping of loci that encode genes important to disease resistance/susceptibility.
- Use of naturally disease and stress resistant animals in production systems.
- Lowered use of antibiotics and vaccines.

Impact

Efficient and profitable animal production systems in which animal well-being is maximized and in which disease is controlled through genetic selection of animals most suited to production schemes.

Research Component 6: Epidemiology of Disease

Goals

Incorporate hypothesis-driven epidemiologic analysis into selected current and future ARS research programs.

Outcome

- Develop an understanding of animal disease incidence and prevalence in livestock and poultry populations.
- Develop understanding of disease life cycles in nature as a means to interrupt disease transmission between livestock and wildlife animals.
- Devise better disease intervention strategies based on epidemiologic observations.
- Identify most effective disease management interventions through comparative on-farm studies.
- Reduce or eradicate key diseases in wildlife that impact livestock production.
- Reduce the incidence of disease in animal production units through use of better management practices, genetics, immune modulation and vaccines.

Impact

More cost-effective livestock production through promotion of animal health and well-being and protection of human health from zoonotic diseases.

Research Component 7: Strategies to Control Infectious and Non-Infectious Diseases

Goals

Develop integrated farm management systems that advance animal health and promote economic viability of rural America.

Maximize the return on limited USDA research resources by developing a prioritized list of livestock and poultry diseases which need the most urgent research and whose control will have the greatest impact on reducing total disease incidence and will provide the maximum economic return.

Identify the needs and the gaps in our basic and applied knowledge concerning control of the highest priority diseases of livestock and poultry.

Develop and integrate basic research information into applied solutions that can be used by farmers and ranchers to control animal diseases in the field. This will include transfer of technology to commercial manufacturers, government agencies and university partners.

Develop comprehensive control strategies that will reduce the incidence and economic losses from endemic, emerging and re-emerging diseases. Eradication strategies will be developed to eliminate some diseases from individual farms, states, regions or the entire country.

Develop strategies to prevent introduction of exotic or foreign animal diseases such as foot-and-mouth disease, classical swine fever and highly pathogenic avian influenza, and develop eradication strategies for rapid elimination of such diseases should they be introduced.

Outcomes

- Development of integrated animal health strategies for the application of new and improved disease control and prevention methods.
- Development of vaccines for diseases that currently have no vaccine.

- Development of more effective (greater efficacy, easier application, and reduced cost) and safer vaccines to replace existing inadequate products.
- Development of improved pharmaceutical products such as antibiotics, antiviral drugs, antifungal compounds, anthelmintics and compounds that kill ectoparasites.
- Development of compounds that reduce or prevent toxic effects of poison plants and other toxins.
- Eradication of some animal diseases in US**.**
- Healthier and more productive animal agricultural industry**.**
- Reduced public health risk from food-borne illness.

Impact

Implementation of control strategies promotes both animal and human health and well-being. Development and maintenance of healthy livestock and poultry populations will provide a low-cost, high quality protein source that promotes health and nutrition to all U.S. citizens, including those of low income and from economically-disadvantaged backgrounds.

PROGRAM IMPACT

Introduction

The basis for determining whether the Animal Health National Program has been successful is to look at its impact. For the purpose this assessment, impact is defined as research that has or will significantly influence the field of science, animal health, American agriculture, and the U.S. economy. Since this is a qualitative assessment that is subject to bias it is critical that a large number of our customers, stakeholders, and partners be given the opportunity to independently review and assess the impact of the program. Accordingly, a survey will accompany this report and will be distributed electronically to reach as many customers, stakeholders, and partners as possible.

The following summarizes the impact of the research conducted during the 2000-2004 Animal Health National Program Cycle. The information to assess the impact of the Animal Health National Program is provided in the appendix of this report. The appendix identifies the 18 research management units and 60 current research information system (CRIS) projects that comprise the Animal Health National Program. The goals, accomplishments, impact, technology transfers, and publications are provided for each of the 60 CRIS projects.

Pathogen Detection and Diagnostics

Anaplasmosis
Anaplasmosis causes disease in cattle on all six continents, being present in South Africa, Australia, Asia, the former USSR, South America, and the United States. Anaplasmosis causes anemia, weight loss, unthriftiness, abortion, and death in affected cattle. Cattle may not exhibit symptoms until they are stressed, such as when calving or when moved to a feedlot. The competitive inhibition Enzyme Linked Immonosorbent Assay (ELISA) test developed by ARS scientists in Pullman, Washington, can be used to detect silent carriers of the bloodborne parasite. This test will allow us to test and ship cattle to Canada and other countries that do not have Anaplasmosis.

Avian Influenza (AI)
A need exists for a rapid and sensitive diagnostic test for the direct detection of both low pathogenic and highly pathogenic avian influenza to support control and eradication strategies during an outbreak in poultry. In collaboration with the National Veterinary Services Laboratory (NVSL), Animal and Plant Health Inspection Service (APHIS), a real time (RT)-PCR test was developed by ARS scientists at the Southeast Poultry Research Laboratory in Athens, GA, which was validated for the detection of any influenza virus and the

subsequent sub-typing of the virus. The test was validated and adopted for clinical use by APHIS and three State veterinary laboratories (Virginia, Pennsylvania, and North Carolina). This diagnostic test has already been used to help control the recent H7N2 influenza outbreak in Virginia and will enhance our nation's ability to rapidly control avian influenza in the event of future outbreaks.

Classical Swine Fever Virus (CSFV)
Rapid identification and diagnosis of a foreign animal disease is critical for successfully controlling and eradicating the disease following introduction. Rapid field-based assays to detect CSFV are not yet available. A rapid real-time fluorogenic hydrolysis probe TaqMan assay was developed by ARS scientists at the Plum Island Animal Disease Center, New York, and evaluated in pigs experimentally infected with a moderately virulent CSF isolate, Haiti-96. The assay was highly sensitive and specific, detecting infected animals up to 5 days prior to the appearance of CSF clinical symptoms. This rapid, real-time RT-PCR assay for CSFV provides a new diagnostic tool that will redefine disease management and control strategies for this highly significant disease.

Chronic Wasting Disease (CWD)
Accurate diagnosis of chronic wasting disease is extremely important for control and eradication. ARS scientists at the Animal Disease Research Unit in Pullman, WA in collaboration with Colorado State University and the Colorado Division of Wildlife, established that the tonsils of deer are a reliable early indicator of infection and established a valid method for detecting this marker in live deer. This test is suitable for use in surveillance of deer in highly populated areas, where hunting is not allowed but artificial feeding may increase disease prevalence. In addition, scientists have established that the dorsal motor nucleus of the vagus nerve is the earliest site of PrP-CWD accumulation in mule deer. This work identifies the tissue to be selected for diagnostic testing in hunter harvest surveys.

Johne's Disease
Johne's disease is a chronic, progressive enteric disease of ruminants caused by *Mycobacterium paratuberculosis*. Improved diagnostic tools for detection of the disease are urgently needed for early detection of subclinically infected animals and reduction of environmental contamination. Scientists at the National Animal Disease Center (NADC), Ames, Iowa, optimized a simple polymerase chain reaction (PCR) assay using DNA in blood and fecal samples for detection of *M. paratuberculosis*. The new DNA sequence of *M. paratuberculosis* has been transferred to a commercial company for development of the assay. This assay will allow early detection of subclinically infected livestock.

Listeria monocytogenes
Research was conducted by ARS scientists at the Animal Disease Research Unit in Pullman, WA to design an assay that allowed a large number of *L. monocytogenes* strains to be serotyped rapidly and inexpensively. As part of a collaborative project between USDA-ARS, Albany, CA and USDA-ARS-ADRU, Pullman, WA, the conventional slide agglutination serotyping assay was converted to an ELISA format. ELISA serotyping of 89 of 101 *L. monocytogenes* isolates agreed with slide agglutination serotyping data, and 100 previously uncharacterized isolates were serotyped unambiguously using the ELISA method. This ELISA assay greatly reduces the time and expense of serotyping L. monocytogenes strains and this will facilitate serotype determination by research and public health labs.

Poult Enteritis Mortality Syndrome (PEMS)

ARS scientists at the Southeast Poultry Research Laboratory (SEPRL), Athens, GA, identified a novel strain of astrovirus isolated from the thymus of turkeys with poult enteritis and mortality syndrome (PEMS) and sequenced the entire genome of the nonhuman strain of astrovirus. ARS researchers then verified that this purified astrovirus caused PEMS-like disease in naive turkey poults as characterized by growth depression, mortality, enteritis, and immunosuppression. A new diagnostic test (RT-PCR) was developed to identify conserved and divergent regions of the astrovirus genome and this test kit was used to detect astrovirus in commercial turkey flocks. These studies indicate an important role of the new astrovirus in PEMS-associated pathology in turkeys. The RT-PCR test was transferred to scientists at Virginia Polytechnical Institute and Texas Veterinary Medical Diagnostic Laboratory and is currently being used to detect astrovirus in commercial flocks. By providing new ways to understand the pathogenesis of astrovirus infection (and PEMS infection) the test will ultimately increase basic knowledge on the interactions between the turkey immune system and virus infections and enable development of a vaccine against turkey PEMS. A rapid and sensitive real-time reverse transcriptase polymerase chain reaction test (RRT-PCR) was subsequently developed to detect turkey astrovirus type-2, one of the major viruses involved in production of PEMS in turkeys. Real-time RT-PCR is a relatively new technology, which offers substantial advantages over the older standard RT-PCR technology, including improved accuracy, speed and sensitivity.

Toxoplasmosis

Toxoplasmosis is the leading cause of blindness in underdeveloped countries. It is also one of the most common causes of parasitic diseases in humans and animals. Pig products are a major source of Toxoplasma gondii infection in the U.S. ARS scientists at Animal Parasite Diseaase Laboratory in Beltsville, Maryland, with funding from the National Pork Producers Council, have developed a sensitive DNA test for the parasite in pig or other animal tissues. The test is very sensitive and specific and will be an important tool for protecting America's food supply and for epidemiological investigations to decrease the numbers of pigs infected with this parasite.

Trichinellosis

The parasitic worm *Trichinella spiralis* contributes to sporadic outbreaks of trichinellosis of humans. Scientists at the Animal Parasite Dsease Laboratory, Beltsville, Maryland, developed a new multiplex PCR test to detect all of the different species of *Trichinella* at the level of a single worm. This new technique has been transferred to the International *Trichinella* Reference Center and the Danish Center of Experimental Parasitology. Being able to identify *Trichinella* species will lead to better control strategies for trichinellosis.

Tuberculosis

In the last seven years, *Mycobacterium bovis* has been isolated from free ranging white-tailed deer in 12 counties in the northern part of the lower peninsula of Michigan. About 5 percent of wild deer, in an area identified as the TB core area, have TB. Tuberculosis has also been found in coyotes, raccoons, a fox, bear, feral cat, elk and bobcat and in 16 beef cattle and two dairy cattle herds in northern Michigan. This is the first reservoir of TB in wildlife identified within the United States and presents significant obstacles to the eradication effort. ARS scientists at the Bacterial Diseases of Livestock Research Unit at the National Animal Disease Center (NADC), Ames, Iowa, have responded to the challenge by developing an indirect blood test that measures nitric oxide induced in mammals in response to *Mycobacterium bovis* infections. The test can detect human, avium, and bovine TB. The advantage of this test is that it can be used in most mammals and only requires one blood sample, and thus the handling of animals once, as opposed to the official standard skin test that requires animals to be handled twice.

Microbial Genomics

Avian Influenza (AI)
ARS scientists at the Southeast Poultry Research Labotaory, Athens, Georgia, have sequenced numerous strains of avian influenza from around the world. They have elucidated the nature and rates of genetic variation of avian influenza viruses in various hosts. We know that the avian influenza viruses are undergoing rapid and continuous mutation events as a natural consequence of adaptation to poultry populations. This rate is slower in the natural hosts, wild waterfowl, but even in these hosts evolution continues to generate novel strains. This information has been useful to basic influenza scientists and has contributed to our fundamental understanding of influenza. In addition, ARS scientists have been able to decode what genetic and biochemical changes occur to make the influenza virus turn into a highly virulent virus.

Bovine Viral Diarrhea Virus (BVDV)
There is a wide spectrum of clinical disease that occurs following BVDV (Bovine Virus Diarrhea Virus) infections. The severity of disease depends on the immune and reproductive status of the animal, the presence of other pathogens and the strain of BVDV that the animal was exposed to. In the past ARS scientists have identified high and low virulence BVDV. In the last cycle, scientists at the USDA-ARS-National Animal Disease Center (NADC), Ames, IA, in collaboration with scientists at the Hannover School of Veterinary Medicine, characterized differences in pathology in vivo, host cell interaction in vitro, and associated genetic differences. They will continue to build on this research as they work to identify the factors that control virulence. They expect that this research will lead to better means of intervention to limit the effects of viral infection. This research has yielded the first published pathogenic studies of high and low virulence BVDV and generated tag sequence libraries for sequential analysis of gene expression (SAGE libraries) in infected cells in vitro. This information will be used to study the interaction of high and low virulence viruses with host cells.

Brucellosis
Brucellosis remains a critical zoonotic pathogen with impact on the reproductive health of cattle, swine, sheep, and goats, and effects on human health. ARS scientists were critical partners this year in supporting a multi-center effort for sequencing the genome of *Brucella suis*, the cause of swine brucellosis. Completing the sequencing of *Brucella suis* is a major accomplishment that will lead to comparative genomics studies that could provide precise targets for developing vaccines against *Brucella* strains that are currently affecting livestock and wildlife reservoir hosts in the United States.

Johne's Disease
ARS scientists at our Bacterial Diseases of Livestock Research Unit at NADC, Ames, Iowa, in collaboration with the University of Minnesota completed the sequencing of the entire genome of *Mycobacterium avium* spp. *paratuberculosis* (*M. paratuberculosis*), the pathogen responsible for Johne's Disease. This bacterium is related to the tubercle bacillus and causes a chronic wasting and debilitating enteritis and eventual death in cattle, sheep, goats, llamas, deer, and other wild or domestic ruminants. Public health officials are also concerned about recently published medical reports suggesting a link between *M. paratuberculosis* and Crohn's disease, a chronic inflammatory bowel disease in humans. ARS scientist have identified 19 unique sequences that will be the basis for functional genomics studies that may eventually lead to the development of new effective diagnostics and preventive and therapeutics tools for the control and eradication of this disease in the

United States. Seven regions of the DNA of *M. paratuberculosis* that stimulate protective responses and one that exacerbates infection have been identified to date. Sequencing was completed on two of the protective regions and a microarray system of gene pooling is being tested. Genes from these regions may be useful in development of a DNA vaccine for *M. paratuberculosis* infection.

Leptospirosis
Little is known about the genetic organization or content of *Leptospira borgpetersenii* serovar hardjo, the major cause of bovine leptospirosis worldwide and an important zoonotic disease. The genome of this bacterium is being sequenced at our Bacterial Diseases of Livestock Research Unit at NADC, Ames, Iowa, to identify genes associated with virulence and potential vaccine candidates. In 2002, a genomic library was successfully constructed and our scientists completed the shotgun phase of sequencing, resulting in the determination of about 95% of the total sequence. These data resulted in the identification of several outer membrane protein genes and genes associated with virulence factors and will have an impact on future vaccine development initiatives.

Marek's Disease
ARS scientists at the Avian Diseases and Oncology Laboratory (ADOL), East Lansing, Michigan, sequenced 175,000 bases of DNA of the GA strain of Marek's disease virus (MDV), a serotype 1 MDV, thus providing a crucial roadmap for understanding viral gene function as an first important step toward better diagnosis of MDV in commercial meat-type chickens. Molecular DNA sequencing of the serotype 1 MDV was transferred to academia and the poultry industry to help the global effort to elucidate the function of various viral genes and the epidemiology of MDV infection. Completion of the entire DNA sequence of the GA strain of MDV will advance the development of novel molecular vaccines for MDV. Furthermore, the DNA sequence information of MDV will enhance understanding of the immunology of MDV infection, and that may prove useful in controlling other types of viruses important to the U.S. poultry industry.

Pox viruses
ARS scientists at our Plum Island Animal Disease Research Center, New York, have sequenced pox virus genes from many different species of animals. The genes of lumpy skin disease, camel pox, avian pox, sheep pox, goat pox and others have been fully sequenced, analyzed and compared. By comparing the genes of many different pox viruses, it has been possible to determine which genes are tightly conserved across the pox virus family. This will enable scientists to learn more about how pox viruses invade the host and cause disease and to develop successful intervention strategies for the future.

Animal Immunology

Immunological Reagents
The accurate assessment of the host immune response to poultry infectious agents such as bacteria, viruses, and parasites is critical for monitoring the health of the flocks. ARS scientists at the Animal Parasite Disease Laboratory, Beltsville, Maryland, produced several monoclonal antibodies to study host immune responses in chickens and have transferred them to diagnostic firms for further development. Several of these monoclonal antibodies can now be used to assess the immune status of chickens vaccinated with different infectious agents. The results to date indicate that these monoclonal antibodies are useful in the identification of lymphocyte subpopulations and macrophages in the tissues and the peripheral blood from chickens infected with microbial agents or vaccinated with the viral vaccines.

Scientists at the Animal Parasitic Diseases Laboratory developed a comprehensive panel of "designer" immune reagents to assess swine immune responses for use in analyses of disease and vaccine studies and neonatal pig development. These include sensitive gene expression tests for >300 swine immune genes, mAb markers for swine cell subsets and immune cytokines, and biotherapeutics. Products from this research will enhance an expanding base of scientific knowledge on mucosal and neonatal immunology of swine, techniques and reagents for veterinary application, practical outcomes for control of zoonotic pathogens and characterization of products with commercial applications.

Coccidiosis
Avian coccidiosis, an intestinal disease caused by intracellular parasites, is estimated to cost the U.S. poultry industry over $600 million annually. Due to the increasing incidence of drug-resistant parasites, drug-independent control strategies are warranted. Scientists at the Animal Parasite Diseases Laboratory, Beltsville, Maryland, identified and genetically engineered a recombinant chicken cytokine molecule called interferon gamma (IFN-γ), which is secreted by T-lymphocytes, and demonstrated its protective effects against coccidian parasites. A U.S. patent issued to the USDA covering several IFN-γ monoclonal antibodies and a sensitive immunoassay to detect chicken IFN-γ is being licensed to a commercial company for worldwide distribution. These antibodies and the assay will allow the assessment of poultry cell-mediated immune responses to vaccines and diseases, facilitating the development of a new generation of immunological control strategies against avian coccidiosis.

Immune Modulators
Novel vaccination strategies using antigen-encoding DNA plasmids have recently been shown to successfully induce protective cellular and humoral immune responses against a variety of infectious diseases. Scientists at our Animal Parasite Disease Laboratory in Beltsville, MD, have documented for the first time in chickens that immune responses induced by DNA vaccination can be enhanced by co-injecting recombinant cytokines or plasmids encoding these cytokines. Administration of recombinant IFN-γ gene decreased coccidia oocyst production, enhanced body weight gain, and improved disease resistance following coccidia challenge infection. The results of this study clearly document that chicken cytokines can be powerful adjuvants providing additional impetus to further investigate their biological activities at the molecular and immunological levels.

Mastitis
Three hundred thousand dairy cows suffer acute endotoxin shock and death annually due to mastitis caused by *Escherichia coli*, which occurs primarily during the early periparturient period. Scientists at our Bovine Functional Genomics Laboratory in Beltsville, Maryland, have identified and characterized the gene for soluble CD14, which binds and neutralizes endotoxins responsible for mastitis. The gene was cloned and recombinant bovine (rbo)-CD14 protein was successfully produced and evaluated. Intraperitoneal injection of rboCD14 together with endotoxin reduced fatality in mice. Preliminary studies indicate that intramammary injection of soluble rboCD14 is 100% effective in preventing mastitis by *E. coli* in lactating dairy cows. This research will potentially lead to a product that for the first time can be used effective to treat and prevent mastitis caused by *E. coli*.

Mechanisms of Disease

Marek's Disease Virus (MDV)
Marek's disease is a lymphocyte proliferation (a type of cancer) and immune suppressive disease of chickens caused by a herpesvirus. Infections with this virus are nearly universal among commercial chicken flocks worldwide. The virus is relatively stable in the environment and is easily spread through the air. Despite widespread use of vaccines, economic losses from mortality of layers and breeders and condemnation of broilers continue to plague producers. Losses tend to be sporadic and unpredictable. Moreover, the virus continues to mutate to greater virulence, potentially reducing the effectiveness of many existing vaccines and causing concerns that vaccines currently considered effective will not protect in the future. Scientists at our Avian Disease and Oncology Laboratory (ADOL) in East Lansing, Michigan, have been conducting functional genomics studies of selected Marek's disease viral genes to better understand the mechanism by which the virus causes changes in infected chickens. Several virus preparations with specific deletions or changes in selected genes were developed and the effects compared to unaltered virus. Results to date indicate that the 132 base pair nucleic acid repeats are nonessential for MDV replication in cell culture and chickens and viruses lacking these repeat sequences are still pathogenic. Furthermore, the putative MDV oncogene called "meq' could be deleted but was found to be essential for the transformation of lymphocytes in infected chickens. It was also found that the pp38 gene in the vaccine virus, Rispens, functions identically to the pp38 gene in the very virulent Md5 virus. Together, these studies are enhancing our knowledge of the mechanisms of virus replication and virulence, which is essential for developing a new class of vaccines against this very important disease.

Pine Needle Abortion
Grazing needles of ponderosa pine, lodgepole pine, common juniper or Monterey Cypress by pregnant cattle results in abortion/premature parturition, retained placentas and endometritis. Substantial economic losses to cattle producers in the western U.S. and Canada occur annually from ponderosa pine needle-induced abortion. A significant accomplishment by ARS scientists has been the isolation and identification of isocupressic acid (ICA) as the abortifacient toxin in pine needles. Furthermore, two ICA derivatives, succinyl and acety ICA were also identified in pine needles and were active abortifacient toxins. ELISA's were developed for ICA and it's sera metabolites using polyclonal antibodies. Using this assay, the absorption into the serum and elimination into the urine of three ICA metabolites was measured in a cow dosed orally with common juniper. Other accomplishments include screening twenty-three tree and shrub species from throughout the western and southern states for abortifacient levels of ICA. Seasonal changes in ICA levels were monitored in ponderosa pine populations in 5 locations. Field and pen studies determined that nutrient status influenced cows to graze pine needles. Similarly, weather patterns had significant impacts on cow's propensity to graze pine needles. This better understanding of what toxins are involved, what species of plant is involved and when the toxin is produced will give ranchers better ability to control the poisoning.

Porcine Reproductive and Respiratory Syndrome (PRRS)
PRRS is a highly infectious disease caused by an RNA virus. Infections in the United States account for up to 15-20% of the yearly economic losses in the swine industry. The vaccines currently on the market have been reported to be relatively ineffective. It is important to understand the reasons for these vaccine failures and the impact on the incidence of disease so that better control strategies can be developed. Scientists at our U.S. Meat Animal Research Center (MARC) in Clay Center, Nebraska, have obtained 18 different PRRS virus isolates from disease outbreaks in the Midwest and sequenced the NSP2 genes of these viruses to examine variations that may correlate with disease expression. Recombinant antigens have been developed and immunity to individual PRRS virus proteins evaluated in sera from naturally infected swine from around the

country. The research team at MARC has conducted kinetic analysis studies of host-cell gene expression in response to PRRS virus infection using cDNA microarrays. Studies to date indicate that there are a number of responsive genes that increase in expression while others decrease in expression as a result of PRRS virus infection. In combination with the sequence analysis of a number of PRRS virus isolates, this research project will lead to an understanding of the host-virus interactions in the field and identification of viral genes responsible for induction of a protective immune response.

Genetic Resistance to Disease

Avian Leukosis Virus (ALV)
Avian Leukosis is a serious threat to the poultry industry since it induces tumors, decreases egg production, and increases mortality in infected chickens. Scientists at the USDA-ARS Avian Disease and Oncology Laboratory, East Lansing, MI, developed a DNA-based test for the ALV receptor gene known as TVB. This genetic test can distinguish if a chicken carries TVB alleles that confer resistant to ALV subgroups B or E, which are some of the most prevalent ALV types in the field. This procedure allows poultry breeders to accurately select for chickens for resistance to specific ALVs, which would enhance

Genetic Diversity Panel
Scientists at our U.S. Meat Animal Research Center (MARC) in Clay Center, Nebraska, have now characterized over 180 single nucleotide polymorphisms (SNPs) in more than 50 bovine genes. These have been used to estimate the genetic diversity in beef cattle with automated SNP assays developed for high-throughput MALDI-TOF MS genotyping. This is significant because DNA sequence variation provides the fundamental material for improving livestock through selection. Our results indicated that once the halotypes for a given gene locus are sufficiently characterized it is possible to correctly assign halotypes to the vast majority of the genotypes in commercial populations of cattle. This allows the evaluation of these alleles as risk factors for infection phenotypes in commercial populations of cattle.

Marek's Disease Virus (MDV)
With Marek's disease costing the poultry industry over $160 million a year, developing methods that augment current vaccinal control methods is a high priority. ARS, in collaboration with the University of Delaware, screened chickens that were disease resistant and susceptible by the new technology of DNA microarrays, which measures expression levels of many genes. Genetic mapping of the differentially expressed genes revealed many positional candidate genes for resistance to Marek's disease. This approach of integrating global expression studies with mapping shows great promise to rapidly identify disease resistance genes, which can be quickly transferred to the poultry industry to enhance disease resistance.

Scrapie
Scrapie is a member of a group of diseases caused by prions that are called Transmissible Spongiform Encephalopathies (TSEs), which includes bovine spongiform encephalopathy (BSE). Scrapie is efficiently transmitted during lambing but other modes of transmission are poorly understood. Scientists at our Virus and Prion Diseases of Livestock Research Unit at NADC, Ames, Iowa, and our Animal Research Unit in Pullman, Washington, initiated transmission studies by describing the distribution and biochemical characteristics of the prion precursor in placenta and fetal tissues and fluids. It was discovered that if the fetus is genetically resistant to disease, the fetal placental cells do not support prion accumulation and it is therefore probable that

transmission by the infected ewe does not occur. These findings will directly impact control and eradication procedures for sheep scrapie in the U.S.

Epidemiology of Disease

Avian Influenza (AI)
Avian influenza, H7N2, has been circulating in the live bird markets (LBMs) of the Northeast since 1994. This virus has the potential for becoming highly pathogenic. ARS scientists at the Southeast Poultry Research Laboratory in Athens, Georgia, in collaboration with the National Veterinary Services Laboratory (NVSL), Animal Plant Health Inspection Service (APHIS), and state officials from New York, New Jersey, and Pennsylvania, have conducted molecular epidemiology studies of viruses coming from the LBMs and have developed a Real Time (RT)-PCR test to rapidly detect birds infected with the influenza virus. The molecular epidemiology studies showed that a single family of viruses has remained in the markets for the last seven years. The RT-PCR test was compared with virus isolations for specificity and sensitivity as part of a validation study for deploying this diagnostic test. The molecular epidemiology data have increased our understanding of this virus and the RT-PCR test when validated will support multi-State influenza eradication efforts in the LBM system.

Avian Pneumovirus (APV)
APV, now classified as an avian metapneumovirus, was isolated from commercial turkeys in Colorado in 1996. The disease was reported in the United Kingdom in 1985 and prior to 1996 the disease was exotic to North America. The virus causes a mild, but rapidly spreading, upper respiratory disease and adverse effect on weight gain and feed conversion. Last year, APV antibody was demonstrated in wild birds in the Southeast U.S. and suggests wild birds could be a source of APV virus transmission to turkeys. Southeast Poultry Research Laboratory scientists in Athens, GA collected swabs from the species of wild birds previously found to have APV antibodies. APV was identified from 12 of the sampled wild birds and it was determined through sequence analysis of the glycoprotein, matrix and fusion genes that the wild bird viruses are closely related to viruses found in domestic poultry. The presence of virus in wild birds and the relatedness to viruses found in domestic poultry indicate the potential for wild birds to spread APV infections in areas of the United States where the virus is currently not found in poultry.

Brucellosis
The ability to epidemiologically trace the origin of brucellosis infections is of great importance for regulatory personnel. At the USDA-ARS-National Animal Disease Center (NADC), Ames, IA, the B. abortus genome sequencing project was used to identify variable regions in the genome in an effort to differentiate B. abortus strains. An assay was developed that allows strains from single origins to be differentiated from other strains such that epidemiologic tracebacks to identify the source of brucellosis infections may now be possible. This assay will be valuable in determining the source of a brucellosis infection in a cattle herd (i.e. wildlife or other cattle) and help in determining if a brucellosis outbreak is from single or multiple sources.

Chronic Wasting Disease (CWD)
CWD is another member of a group of diseases called TSEs, which includes BSE and Scrapie. CWD is associated with serious losses to the captive elk industry and the wildlife management industries through loss of hunting revenues. The only choice currently available to animal health officials for controlling this disease

is extensive depopulation of captive and free ranging animals exposed to the disease. Moreover, the interspecies transmission barriers of these diseases are not understood. CWD is reported in several areas of the U.S. but the true extent of the epidemic is not known because of the considerable cost of diagnostic testing. Using samples submitted by cooperating state wildlife management agencies, ARS scientists at the Animal Research Unit in Pullman, Washington, developed an inexpensive high throughput diagnostic test for early detection of infection in hunter-killed deer. The test has been transferred to private industry and is being evaluated for licensing by the USDA Center for Veterinary Biologics. The impact of this work will be to facilitate increased surveillance within and outside the endemic areas.

Malignant Catarrhal Fever (MCF)
The epidemiology of sheep-associated Malignant catarrhal fever has long remained an area of great uncertainty. Scientists at the Animal Disease Research Unit in Pullman, WA, Collaborating with Washington State University and the ARS-US Sheep Experiment Station developed a refined picture of the viral shedding patterns in both adolescent and adult sheep. The data indicate that nasal shedding is the major mode of sheep-associated MCF virus transmission among domestic sheep and that the adolescents are by far the heaviest shedders of virus for other species. The kinetics of the shedding patterns documented in adolescent sheep appear to be a unique biological phenomenon without precedent and definition of the virus-host mechanisms underlying this behavior should lead to new knowledge of the potential ways viruses can interact with hosts.

Neosporosis
Neospora caninum has been identified as a major cause of abortion and neonatal mortality in cattle worldwide. Losses in California dairies due to neosporosis-associated abortions are estimated to be $35 million annually. Epidemiological investigations indicate that up to 45 percent of abortions in dairy cows are caused by *N. caninum*. ARS scientists discovered that the dog was the definitive host and the source of new infections. ARS investigated the prevalence of *N. caninum* antibodies in dogs and established that levels were significantly higher in dogs from beef and dairy cattle farms than dogs from urban areas. However, recent reports suggest that congenital infection from reactivation of the parasite is the main mode of transmission and cause of abortions. Considerable work remains to understand the biology and epidemiology of *N. caninum,* including the survival of oocysts in the environment and elucidating the life-cycle in dogs. ARS will continue to conduct studies that will provide information to help dairy farmers develop biosecurity measures to control abortions due to Neosporosis.

Tuberculosis
Bovine tuberculosis is considered a public health threat because humans can become infected with the infectious agent, *Mycobacterium bovis*, through contact with infected animals or contaminated foods. ARS scientists at the National Annimal Disease Center (NADC), Ames, Iowa, demonstrated that white-tailed deer populations living in northeastern Michigan were the source of tuberculosis infection of livestock. These results will facilitate development of new eradication control strategies to block the transmission of *M. bovis* from wildlife to domestic livestock and thereby reduce the possibility of human disease.

Vesicular Stomatitis Virus
Vesicular stomatitis virus (VSV) from an unknown source caused great economic losses to the cattle and horse industries in the southwestern United States in 1995, 1997, and 1998. Using genomic sequencing and analysis, ARS scientists at Plum Island, New York, demonstrated that the outbreaks resulted from VSV most similar to those circulating in Mexico. The new molecular test for VSV field strains has been used by a USDA-APHIS-supported diagnostic laboratory for vesicular disease studies in South American countries.

Strategies to Control Infectious and Non-Infectious Disease

Foot-and-Mouth Disease Virus (FMD)
Current models and simulations have predicted devastating economical and environmental consequences if FMDV were introduced in the United States. Based on recent experiences with FMDV outbreaks in Europe, vaccination is now being considered as a first-line intervention tool in the containment of a potential outbreak in the U.S., rather than traditional depopulation strategies that would result in destruction of millions of animals. Scientist at our FMDV Research Unit at the Plum Island Animal Disease Center, New York, have developed adenoviral based constructs containing the structural proteins of the FMDV capsid and the viral protease required to process these protein molecules into empty capsids. Laboratory tests of these viral vaccines have demonstrated virus neutralizing activity and protection of animals as early as five days after a single inoculation, lasting for at least 42 days. The same adenoviral vectors have been engineered to contain antivirals such as Type I porcine interferon (Ad5-pIFNalpha) and have been shown to confer protection from viral challenge as early as 24 hours and for as long as four days after a single inoculation. The combination of empty capsid vaccines and antivirals have been tested in swine and shown to induce complete protection from infection in 24 hours and for as long as 42 days upon direct challenge with FMDV. This combined therapy provides for the first time a true outbreak response strategy to arrest the spread of FMDV and limit animal exposure to this highly contagious disease. Further work will be needed to research and develop these innovative control tools for other animal species, including cattle.

Milk Fever
Scientists at the Periparturient Diseases of Livestock Research Unit, National Animal Disease Center (NADC), Ames, Iowa, fed various sources of chloride and sulfate containing chemicals to dry non-pregnant dairy cows and demonstrated that chloride consistently counteracted the effects of dietary potassium by causing a decrease in blood acidity which contributes to hypocalcemia (milk fever). This new information has resulted in the development of hydrochloric acid treatment of feeds by at least two feed companies (West Central Corp., Ralston, Iowa, and Nutritech, Abbottsford, British Columbia) and allowed farmers, veterinarians, and nutritionists to more accurately choose the most effective feeds and chloride sources for balancing transition dairy cow rations. These results will also be applicable to the inability of dairy cows to respond to the high demand for calcium at parturition and ultimate development of peri-parturient hypocalcemia. This research has provided a more detailed understanding of the pathogenesis of milk fever. It has emphasized the importance of dietary methods for reversing alkalosis in order to reestablish the target tissue sensitivity to parathyroid hormone and to prevent clinical and sub-clinical hypocalcemia in dairy cows. An enhanced understanding of the effects of dietary factors on the overall health of young and adult dairy cattle will benefit U.S. producers and consumers.

Scientists at the Periparturient Diseases of Livestock Research Unit have also investigated the utility of *Solanum glaucophyllum* in the prevention of subclinical hypocalcemia in periparturient dairy cows. *Solanum glaucophyllum* is a plant that contains the active form of vitamin D. ARS scientists discovered that when used in combination with diets high in anions (chloride), we can significantly reduce the incidence of subclinical hypocalcemia in dairy cows. Availability of this plant will offer further opportunities to dairy farmers in avoiding complications due to hypocalcemia such as displaced abomasum, retained placenta, and mastitis.

Mycoplasma gallisepticum (MG)

Approximately 90% of commercial layer flocks are infected with *Mycoplasma gallisepticum* (MG); and to combat its effects, three live MG vaccines are available for use; however, MG vaccine administration has become a major problem from the perspective of uniformity and consistency. Research into the development of a self-propelled vaccinator capable of vaccinating 75,000 chickens in 7½ minutes was conducted at the Mid-South Area Poultry Research Unit at Mississippi State, MS and involved collaborators from the commercial table egg company Cal-Maine Foods, Inc., Jackson, MS. The research has resulted in a machine which is capable of uniformly and consistently delivering MG vaccine to 75,000 chickens in less than 8 minutes as compared to 50 minutes while reducing labor by 60%. The impact of this research is that producers using the available commercial vaccines in conjunction with this vaccinator can administer MG vaccines quicker and more consistently and uniformly to pullets.

Newcastle Disease

Newcastle disease is a foreign animal disease that has the potential to devastate the poultry industry. The capability to easily differentiate vaccinated from infected birds would enhance current Newcastle Disease control programs. At the Southeast Poultry Research Laboratory in Athens, GA, a vaccine was developed that is a shell of the whole virus and retains the important surface structures that induce immunity. The virus used in this vaccine cannot infect or be transmitted from bird to bird because the virus genome and internal proteins have been removed. This "virosome" Newcastle disease vaccine was administered intra-nasally and shown to protect chickens against a lethal Newcastle disease virus challenge. Because vaccinated birds can be differentiated from infected birds, this vaccine may provide an effective tool to control future outbreaks of Newcastle disease virus in the United States.

Swine Influenza Virus (SIV)

SIV is a serious respiratory disease in pigs. Inactivated and/or modified-live vaccines for respiratory pathogens do not stimulate protective immunity if given to piglets too early before maternally-derived antibodies have decayed. To overcome this problem, scientists at USDA-ARS-National Animal Disease Center (NADC), Ames, IA, used a recombinant human adenovirus (rAd5) vaccine in combination with an inactivated commercial vaccine to stimulate protective immunity in piglets that had natural field exposure levels of maternally-derived antibodies. Piglets suckling gilts that were naturally infected with H3N2 influenza virus were primed with the rAd5-swine influenza virus (SIV) vaccine or sham-inoculated with a non-expressing rAd5 vector at 7 days of age. The sham-inoculated group and half of the rAd5-SIV vaccinated pigs were boosted with a commercial bivalent vaccine when 4 weeks old. The boosted pigs that had been primed in the presence of maternal interfering antibody had strong anamnestic immunity. But, like the problems encountered in the field, the sham-inoculated pigs did not respond to the commercial vaccine because of interfering maternal antibodies. Two weeks after the commercial booster vaccination the pigs were challenged with a different, virulent H3N2 influenza virus. The efficacy of the vaccination protocol was demonstrated by abrogation of clinical signs, by clearance of challenge virus from lung lavage fluids, by markedly reduced virus shedding in nasal secretions, and by the absence of moderate or severe SIV-induced lung lesions. These rAd5-SIV vaccines will be used for priming of the immune system to overcome the effects of maternally-derived antibody which interferes with conventional SIV vaccines.

Appendix

Research Sites
Research Units
CRIS Projects

- Objectives
- Accomplishments
- Impact
- Technology Transfers
- Publications

Plum Island Disease Center
Greenpoint, NY

Foot and Mouth Disease Research
Rodriguez, Luis L
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African Swine Fever Research
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PROJECTS

Foot and Mouth Disease Research

- Development of Novel Strategies to Control Foot-and-Mouth Disease
- Determination of the Molecular Basis for Re-Emergence, Pathogenesis, And Host Range of FMDV
- Pathogenesis and Genomics of Vesicular Stomatitis Viruses and Foot and Mouth Disease

African Swine Fever Research

- High Consequence Pathogen Detection
- Functional Pathogen Genomics: Virulence and Host Range Genes of Swine Fever Virus
- Identification and Characterization of African Swine Fever Virus Protective Antigens
- Detection and Diagnosis of Hog Cholera (CSF)
- Advanced Animal Vaccines and Diagnostic Applications
- Advanced Veterinary Vaccines and Diagnostics
- Program for Prevention of Animal Infections and Advanced Technologies for Vaccines and Diagnostics
- Development of Rapid Real Time PCR-Based Assays for Selected OIE Class A Diseases

Foot and Mouth Disease Research

Project Title: Development of Novel Strategies to Control Foot-and-Mouth Disease
CRIS Number: 1940-32000-034-00D
Scientists: Grubman, M., Golde, W., Baxt, B., and Gregg, D.
Location: Plum Island Animal Disease Center, Office of the Center Director, Foot and Mouth Disease research
Contact: Grubman, M., Phone: (631) 323-3329, Fax: (631) 323-3006
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PROJECT OBJECTIVES:
Foot-and-mouth disease (FMD) is a highly contagious viral disease of livestock. FMD virus (FMDV), the causative agent, spreads quickly between animals, and herds, resulting in devastating epizootics and significant economic hardship. Normally controlled by slaughter and vaccination with chemically inactivated virus, concerns with the effectiveness of this vaccine and the fact that Federal law prohibits its production in the US creates a significant need for genetically engineered vaccines as potential alternatives. **We are evaluating the basis for protective immunity and developing new vaccines that are safer and more effective than traditional products**. To investigate non-traditional control strategies, we are obtaining detailed knowledge of virus-host cell interactions and the immune response to viral infection. Our approach towards these objectives includes examining the means by which the virus inhibits the initial host defense response and developing subunit vaccine candidates that do not contain infectious material but induce protective immunity. These latter products could be produced in the US since they do not contain infectious FMDV. In addition, we are developing new knowledge of the immune response to FMDV and FMD vaccines to improve this vaccine.

OVERALL PROJECT ACCOMPLISHMENTS:
Demonstrated that the FMDV L nonstructural protein is a major virulence factor in viral pathogenesis allowing for a better understanding of virus-host interaction.

Developed novel subunit vaccine candidates including naked DNA, and replication-defective human adenovirus as vectors for delivery of FMDV capsid antigens. These vaccine candidates have a number of advantages over traditional chemically inactivated FMD vaccines including the possibility of producing them in the US.

Demonstrated that one inoculation of a replication-defective human adenovirus containing the capsid coding region of FMDV serotype A24 can completely protect swine from direct inoculation challenge as early as 7 days and as long as 42 days. In preliminary studies in cattle, two doses of the same vaccine can induce complete protection from direct inoculation as well as contact challenge.

Developed a model of acute infection in swine for analysis of the initiation of the innate and adaptive immune response to viral infection.

Demonstrated immune cell dysfunction during acute phase infection correlates with interruption of specific cytokine production.

Developed a method to isolate skin derived dendritic cells for analysis of the role of these cells in innate immunity and initiation of adaptive immunity. Showed that these cells constitutively express IFNα in situ.

Demonstrated that one inoculation of a recombinant adenovirus vector containing the gene for porcine interferon alpha (Ad5-pIFNalpha) can protect swine from FMDV challenge one day post inoculation.

Demonstrated that the combination of IFN alpha administration and vaccination with our Ad5-vectored FMDV capsid antigen construct can induce both immediate as well as long term protection.

IMPACT:
The development of a adenovirus vectored combination vaccine and antiviral against FMDV that provides immediate protection in 1 day through its antiviral activity and long-term protection through its FMD empty capsid vaccine has had a major impact on the world view of FMD outbreak control. This has resulted in recommendations favoring vaccination over mass slaughtering of millions of animals. The Ad5 vaccine is the first genetically engineered vaccine against FMDV shown to induce full protection in swine with one dose as early as 7 days post vaccination. Preliminary studies in cattle indicated that two doses of this vaccine can induce complete protection and serological data after the first inoculation suggest that protection can be induced after only one dose. These vectors can be produced in the US and be part of the US response in the event of an FMD outbreak in this country.

TECHNOLOGY TRANSFER:
Our research studies have been published in peer-reviewed scientific journals. Genetically engineered vaccine candidates have been produced, and have been proven effective in livestock (swine and bovine) but they have not undergone final development.

Patents:
We have filed a Patent concerning some of our new concepts to control FMD.
Foot and Mouth Disease Virus Vaccine, Grubman, Marvin J., Chinsangaram, Jarasvech, Koster, Marla, Moraes, Mauro, P. Patent, Docket Number:0128.01, Serial Number: 60/286, 345, Date Filed: 4/26/2002.

Cooperative Research Development Agreements (CRADA):
We have entered into a CRADA with Merial Limited to produce antivirals using poxvirus vectors as a means of inducing immediate protection against FMD as well as an SCA with Merial to produce poxvirus vectored FMDV empty capsid vaccines. We have entered an SCA with the University of Connecticut at Storrs to study the immune response and pathogenesis of FMDV and VSV in swine. We have entered into a second SCA with the University of Connecticut at Storrs for testing rapid acting FMDV vaccine formulations in cattle. We have a MTA with Qiagen to test their exclusive immune stimulator compounds in combination with commercial FMD vaccines.

PUBLICATIONS:

Brown, C.C., Chinsangaram, J., and Grubman, M.J. Type I interferon production in cattle infected with 2 strains of foot-and-mouth disease virus, as determined by in situ

hybridization. Canadian J. Vet. Res. 64:130-133, 2000.

Grubman, M.J. and Chinsangaram, J. Foot-and-mouth disease virus: The role of the leader proteinase in viral pathogenesis. Recent Res. Devel. Virol, 2:123-134, 2000.

Mayr, G.S., O'Donnell, V., Chinsangaram, J., Mason, P.W., and Grubman, M.J. Immune responses and protection against foot-and-mouth disease virus (FMDV) challenge in swine vaccinated with adenovirus-FMDV constructs. Vaccine 19:2152-2162, 2001.

Chinsangaram, J., Koster, M., and Grubman, M.J. Inhibition of L-deleted foot-and-mouth disease virus replication by alpha/beta interferon involves double-stranded RNA -dependent protein kinase. J. Virol. 12:5498-5503, 2001.

Mason, P.W. and Grubman, M.J. Controlling FMD with vaccines? Australian Vet. J. 79:342-343, 2001.

Moraes, M.P., Mayr, G.A., and Grubman, M.J. pAd5-Blue: an easy to use, efficient, direct ligation system for engineering recombinant adenovirus constructs. BioTechniques. 31:1050-1056. 2001.

Moraes, M.P., Mayr, G.A., Mason, P.W., and Grubman, M.J. Early protection against homologous challenge after a single dose of replication-defective human adenovirus type 5 expressing capsid proteins of foot-and-mouth disease virus (FMDV) strain A24. Vaccine. 20:1631-1639, 2002.

Grubman, M.J. and Mason, P.W. Prospects, including time frames, for improved foot -and-mouth disease vaccines. *In* Foot and mouth disease: facing the new dilemmas. Rev. sci. tech. Off. Int. Epiz. 21:589-600. 2002.

Bautista, E., D. Gregg, and W.T. Golde. Characterization and functional analysis of skin-derived dendritic cells from swine without a requirement for in vitro propagation. Veterinary Immunology and Immunopathology. 87: 137-145, 2002.

Mason, P.W., Grubman, M.J., and Baxt, B. Molecular basis of pathogenesis of FMDV. *In* Foot-and-mouth disease (D. Rowlands ed.). Virus Res. 91:9-32, 2003.

Chinsangaram, J., Moraes, M.P., Koster, M. and Grubman, M.J. A novel viral disease control strategy: adenovirus expressing interferon alpha rapidly protects swine from foot -and-mouth disease. J. Virol. 77:1621-1625, 2003.

Grubman, M.J., Moraes, M.P., Chinsangaram, J., Mayr, G.A., and Mason, P.W. New approaches to control foot-and-mouth disease, p. 337-343. Foot and mouth disease: Control strategies. (B. Dodet and M. Vicari eds). Elsevier. 2003.

Mason, P.W., Chinsangaram, J., Moraes, M.P., Mayr, G.A., and Grubman, M.J.

Engineering better vaccines for foot-and-mouth disease. Vaccines for OIE List A and Emerging Animal Diseases. Dev. Biol. Basel, Karger (Brown, F, Roth, J eds). 114:79-88, 2003.

Wu, Q., Moraes, M.P., Grubman, M.J. Recombinant adenovirus co-expressing capsid proteins of two serotypes of foot-and-mouth disease virus (FMDV): in vitro characterization and induction of neutralizing antibodies against FMDV in swine. Virus Research. 2003. v. 93. p. 211-219.

Bautista, E., Ferman, G.S., Golde, W.T. Induction of lymphopenia and inhibition of T cell function during acute infection of swine with foot and mouth disease virus (FMDV). Veterinary Immunology and Immunopathology. 2003. v. 92. p. 61-73.

Wu, Q., Brum, M.C.S., Caron, L., Koster, M., and Grubman, M.J. Adenovirus-mediated type I interferon expression delays and reduces disease signs in cattle challenged with foot-and-mouth disease virus. J. Int. Cyt. Res., 23:371-380, 2003.

Grubman, M.J. New approaches to control foot-and-mouth disease. Proceedings of the International Symposium on Foot-and-Mouth Disease, Strasbourg, France, pp. 83-87, 2003.

Moraes, M.P., Chinsangaram, J., Brum, M.C.S., and Grubman, M.J. Immediate protection of swine from foot-and-mouth disease: a combination of adenoviruses expressing interferon alpha and a foot-and-mouth disease virus subunit vaccine. Vaccine. 22:268-279, 2003.

Grubman, M.J. New approaches to rapidly control foot-and-mouth disease outbreaks. Expert Rev. Anti-infect. Ther. 1:89-96, 2003.

Rodriguez, L. L., J. Barrera, E. Kramer, J. Lubroth, A. Walfield, F. Brown and W. T. Golde. A synthetic peptide containing the consensus sequence of the G-H loop region of FMDV type O VP1 combined with a promiscuous T-helper epitope induces peptide-specific antibodies but fails to protect cattle against viral challenge. Vaccine 21: 3751-3756, 2003.

Foot and Mouth Disease Research

Project Title: Determination of the Molecular Basis for Re-Emergence, Pathogenesis and Host Range of FMDV
CRIS Number: 1940-32000-035-00D
Scientists: Baxt, B., Rieder, E., Grubman, M., Golde, W., Gregg, D.
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PROJECT OBJECTIVES:
Foot-and-mouth disease (FMD) is a highly contagious viral disease of livestock. The causative agent, foot-and-mouth disease virus (FMDV), has been extensively studied due to its economic impact. Recently, FMD has re-emerged in Europe, South America and Asia with devastating consequences. This re-emergence has emphasized how little is known about the detailed virus-host interactions in different susceptible livestock species that control the pathogenesis and virulence of the virus. This information is necessary to develop new strategies to assist in preventing FMD epizootics. **The Plum Island Animal Disease Center (PIADC) performs research developing such strategies to assist in preventing the introduction of FMD and to control it if it is introduced.** As part of this program, this project will determine the virus-host interactions at the cellular and molecular levels. These interactions, critical in the replication cycle and spread of FMDV, start with the interaction between the virus and receptor molecules on susceptible cells and follow intracellular pathways of viral replication and mechanisms of viral exit. We will use this information to find targets for novel anti-viral therapeutics and means to prevent infection of susceptible animals. In addition, knowledge of the genetic basis of viral transmission and spread will be obtained to help us better cope with the disease, if it is introduced into the United States.

OVERALL PROJECT ACCOMPLISHMENTS:
- Molecular cloning and characterization of all cell surface molecules from the bovine that serve as receptors for FMDV.
- Identification of at least one viral (3A protein), and host (ITAF45) factor that play roles in determining virulence and species specificity.
- Development of models to measure FMDV virulence and spread as it relates to the genetic makeup of the virus.
- Analysis of the efficiency of utilization of the different receptor molecules by the different viral serotypes.
- Generation of soluble FMDV receptors which can neutralize viral infectivity.

IMPACT:
The identification of the receptors for FMDV has enhanced our ability to study the pathogenesis of the disease. In addition, the cloning of these receptors has allowed us to study new ways of using these molecules in rapid diagnosis. Data showing that soluble receptors can neutralize viral infectivity suggest that receptor intervention using antivirals may be a feasible way to interfere with viral replication in susceptible animals. The identification of viral and host cell proteins important for FMDV virulence and host specificity has identified important new targets for anti-viral therapeutics.

TECHNOLOGY TRANSFER:
Transfer of a genetically improved cell line to APHIS that allows more effective isolation of foot-and-mouth disease virus (FMDV). A hamster cell line (BHK-21) frequently used for the diagnosis of FMDV, was engineered to increase its susceptibility to FMDV by expressing the integrin alphaVbeta3 and alphaVbeta6 receptors, previously discovered at Plum Island as one of the main cellular receptors for FMDV.

PUBLICATIONS:

Neff, S., Mason, P. W., and Baxt, B. High efficiency utilization of the bovine integrin $\alpha_V\beta_3$ as a receptor for foot-and-mouth disease virus is dependent on the bovine β_3 subunit. J. Virol. 74:7298-7306. 2000.

Neff, S., and Baxt, B. The ability of the integrin $\alpha_V\beta_3$ to function as a receptor for foot-and-mouth disease virus is not dependent on the presence of complete subunit cytoplasmic domains. J. Virol. 75:527-532. 2001.

O'Donnell, V. K., Pacheco, J. M., Henry, T. M., and Mason, P. W. Subcellular distribution of the foot-and-mouth disease virus 3A protein in cells infected with viruses encoding wild-type and bovine-attenuated forms of 3A. Virology 287:151-162. 2001.

Baxt, B., Neff, S., Rieder, E., and Mason, P. W. Foot-and-mouth disease virus-receptor interactions: Role in pathogenesis and tissue culture adaptation. In Semler, B. L., and Wimmer, E. (eds.). Molecular Biology of Picornaviruses. ASM Press, Washington, DC. pp. 115-123. 2002.

Van Rensburg, H. G., and Mason, P. W. Construction and evaluation of a recombinant foot-and-mouth disease virus: implications for inactivated vaccine production. Ann. NY Acad. Sci. 969:83-87. 2002.

Mason, P. W., Bezborodova, S.V., and Henry, T. M. Identification and characterization of a cis-acting replication element (cre) adjacent to the internal ribosome entry site of foot-and-mouth disease virus. J Virol. 76: 9686-9694. 2002.

Duque, H., and Baxt, B. Foot-and-mouth disease virus receptors: Comparison of bovine alphaV integrin utilization by type A and O viruses. J. Virol. 77:2500-2511. 2003.

Mason, P. W., Grubman, M. J., and Baxt, B. Molecular basis of pathogenesis of FMDV. Virus Res. 91:9-32. 2003.

Zhao, Q., Pacheco, J. M., and Mason, P. W. Evaluation of genetically engineered derivatives of a Chinese strain of foot-and-mouth disease virus reveals a novel cell-binding site which functions in cell culture and in animals. J. Virol. 77:3269-3280. 2003

Mason, P. W., Pacheco, J. M., Zhao, Q. Z., and Knowles, N. J. Comparison of the complete genomes of Asian, African, and European isolates of a recent foot-and-mouth disease virus type O pandemic strain (PanAsia). J. Gen. Virol. 84:1583-1593. 2003.

Mason, P. W., Chinsangaram, J., Moraes, M. P., Mayr, G. A., and Grubman, M. J. Engineering better vaccines for foot-and-mouth disease. Dev. Biol. 114:79-88. 2003.

Pacheco, J. M., Henry, T. M., O'Donnell, V. K., Gregory, J. B., and Mason P. W. Role of nonstructural proteins 3A and 3B in host range and pathogenicity of foot-and-mouth disease virus. J. Virol. 77: 13017-13027. 2003.

van Rensburg, H. G., Henry, T. M., and Mason, P. W. Studies of genetically defined chimeras of a European type A virus and a South African Territories type 2 virus reveal growth determinants for foot-and-mouth disease virus. J. Gen. Virol. 85: 61-68. 2004.

Grubman, M. J., and Baxt, B. Foot-and-mouth disease. Clin. Microb. Rev. 17:465-493. 2004.

Baxt, B., and Rieder, E. Molecular Aspects of foot-and-mouth disease virus virulence and host range: Role of host cell receptors and viral factors. In Sobrino, F., and Domingo, E. (eds.). Foot-and-Mouth Disease: Current Perspectives. Horizon Scientific Press, in press, 2004.

Duque, H., LaRocco, M., Golde, W. T., and Baxt, B. Interactions of foot-and-mouth disease virus with soluble bovine $\alpha_V\beta_3$ and $\alpha_V\beta_6$ integrins. J. Virol., in press, 2004.

Foot and Mouth Disease Research

Project Title: Pathogenesis and Genomics of Vesicular Stomatitis Viruses and Foot and Mouth Disease
CRIS Number:: 1940-32000-040-00D
Scientists: Rodriguez, L., Golde, W., Gregg, D.
Location: Plum Island Animal Disease Center, Office of the Center Director
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PROJECT OBJECTIVES:
Vesicular stomatitis (VS) is a viral disease that causes serious economic losses to the cattle and horse industries due to decreased animal production and quarantines. The disease is clinically indistinguishable from foot-and-mouth disease, one of the most devastating exotic diseases in livestock. VS is the most commonly reported vesicular disease in livestock in the Americas and is included as a List-A disease by OIE. Outbreaks of VS occur sporadically in the south-western United States. VS virus (VSV) strains causing these outbreaks are closely related to those circulating in enzootic areas of Mexico but their means of transmission and introduction to the United States remain unclear. Other exotic VS viruses circulate in South America. Knowledge about these viruses is limited and rapid sensitive and specific diagnostic tests for VSV are lacking. Host and viral factors influencing clinical presentation and mediating protective immune responses to VSV are poorly understood. This information is required in order to design appropriate prevention and outbreak control methods. This project has two separate components both aimed at minimizing the occurrence of VS in the US: **Objective 1 utilizes viral genomics for diagnosis and molecular epidemiology, to rapidly detect and track the origin of VSV strains causing outbreaks. Objective 2 addresses pathogenesis aspects specifically the role of viral and host factors in determining the outcome of viral infections.** This could result in the development of therapeutic agents capable of preventing clinical disease during outbreaks.

OVERALL PROJECT ACCOMPLISHMENTS:
ORIGIN OF VESICULAR STOMATITIS OUTBREAKS IN SOUTH WEST.
Using genomic analyses, determined that viruses causing the 1995, 1997 and 1998 VS outbreaks were newly emerging viruses, they were different from viruses causing previous US outbreaks but similar to viruses circulating in enzootic areas of southern Mexico. This information is valuable in focusing our attention on the factors influencing the introduction of VSV into the US.

FIRST FULL-LENGTH GENOMIC SEQUENCES OF VSV FIELD STRAINS. Full length genomic sequences were determined for each of the major serotypes and subtypes of VSV. These sequences provided crucial information for understanding the genomic basis of viral pathogenesis and allowed the identification of genetic signatures for diagnostic assay development, such the VSV rapid real-time RT-PCR.

ROLE OF THE GLYCOPROTEIN IN PATHOGENESIS OF VESICULAR STOMATITIS IN LIVESTOCK.
Host and viral factors influencing clinical presentation and mediating protective immune responses to VSV are poorly understood. This information is required in order to design appropriate prevention and outbreak control methods. In collaboration with the University of Alabama-Birmingham, we showed that the VSNJV glycoprotein is a determinant of pathogenesis in swine. Furthermore, genetically engineered VSVs containing

glycoproteins from both serotypes induced immune responses to both VSNJV and VSIV but could only protect against VSIV challenge. This information is valuable for designing effective vaccines.

IMPACT:
Phylogenetic analyses of VSV strains combined with epidemiological information provided valuable tools for understanding the molecular epidemiology of VSV in the United States. Full-length genomic information provide de the basis for the design of rapid detection methods for VSV based on real-time RT-PCR. This rapid diagnostic test currently under validation in APHIS, is part of a National strategy for developing real-time RT_PCR test for rapid detection of foreign animal disease agents. Identifying the glycoprotein gene as a virulence determinant for VSV has had a major impact in understanding the protective immune responses to VSV.

TECHNOLOGY TRANSFER:
Material Transfer Agreement
ONE-TUBE RAPID DIAGNOSTIC TEST FOR VESICULAR STOMATITIS. This test based on real-time PCR was designed to detect a wide range of vesicular stomatis virus strains from various geographical regions of the Americas. The test has been transferred to APHIS-FADDL for ongoing validation and will be transferred to other regional laboratories of the National Animal Health Laboratory Network during 2004.

PUBLICATIONS:

Rodriguez, L L, Bunch T A, Fraire M, and Llewellyn Z. 2000. Re-emergence of vesicular stomatitis in the western United States is associated with distinct viral genetic lineages. Virology 271: 171-181.

Flanagan, E. B., J. M. Zamparo, L. A. Ball, L. L. Rodriguez, and G. W. Wertz. 2001. Rearrangement of the genes of vesicular stomatitis virus eliminates clinical disease in the natural host: new strategy for vaccine development. Journal of Virology 75: 6107-6114.

Rodriguez L L. 2002. Emergence and Re-Emergence of Vesicular Stomatitis in The United States. Virus Research 85: 211-219

Rodriguez, L.L., S.J. Pauszek, T. A. Bunch and K.R. Schumann. 2002. Full-Length Genome Analysis of Natural Isolates of Vesicular Stomatitis Virus (Indiana 1 Serotype) from North, Central and South America. Journal of General Virology 83: 2475-2483.

Llewellyn, Z., M.D. Salman, S. J. Pauszek, and L.L. Rodriguez. 2002. Growth and Molecular Evolution of Natural Isolates of Vesicular Stomatitis Virus Serotype New Jersey in Insect Cells. Virus Research 89: 65-73.

Meissner F., Maruyama T., Frentsch M., Hessell A. J. Rodriguez L.L., Geisbert T.W., Jahrling P.B., Burton D.R., and Parren W.H.I. 2002. Detection of Antibodies against the Four Subtypes of Ebola Virus in Sera from Any Species Using a Novel Antibody-Phage Indicator Assay. Virology 300: 236-243

Martinez I., Rodriguez L.L., Jimenez C., Pauszek S.J. and Wertz G.W. 2003. Vesicular Stomatitis Virus Glycoprotein is a Determinant of Pathogenesis in Swine, a Natural Host. Journal of Virology 77: 8039-47.

Rodriguez L.L., Barrera J.C., Kramer E., Lubroth J., Brown F., and Golde W.T. 2003. A Synthetic Peptide Containing The Consensus Sequence Of The G-H Loop Region Of FMDV Type O VP1 Combined With A Promiscuous T-Helper Epitope Induces Peptide-Specific Antibodies But Fails To Protect Cattle Against Viral Challenge. Vaccine 21: 3751-3756.

Magnuson RJ, Triantis J, Rodriguez LL, Perkins A, Meredith CO, Beaty B, McCluskey B, Salman M. 2003. A single-tube multiplex reverse transcription-polymerase chain reaction for detection and differentiation of vesicular stomatitis Indiana 1 and New Jersey viruses in insects. Journal of Veterinary Diagnostic Investigations. v15 p. 561-567

African Swine Fever Research

Project Title: High Consequence Pathogen Detection
CRIS Number:: 1940-32000-041-02S
Scientists: Rock, D., Huxsoll, D., McKenna, T., Adams, L.
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PROJECT OBJECTIVES:
Although exotic livestock diseases, Foot-and-Mouth Disease Virus (FMDV), Classical Swine Fever (CSF), African swine fever (ASF), rinderpest (RP), contagious bovine pleuropneumonia (CBPP) and lumpy skin disease (LSD)may enter the United States mainland by various means, the potential for terrorists, seeking vulnerable or soft targets in the US, to deliberately introduce foreign animal diseases has added a new dimension to livestock diseases as a threat to the national economy. Foreign animal diseases (FAD) are a likely choice for terrorists in that many of the disease agents are easy to acquire, dissemination is not difficult, the diseases are readily transmissible, the livestock population is highly susceptible, and the economic impact of a major outbreak would be catastrophic.

To counter the new threat concerns the U.S. Department of Agriculture is developing a system of rapid real-time PCR assays for selected OIE class A animal pathogens to enable rapid detection and identification of diseased animals and effective management of disease outbreaks resulting from accidental or intentional introduction into the United States. This report serves to document the accomplishments of this project for fiscal year 03.

OVERALL PROJECT ACCOMPLISHMENTS:
The major accomplishments over the life of this project include the design, development, optimization, and bench validation of a "dried-down" real-time RT-PCR assay for FMDV and CSFV. The FMDV and CSFV assays are ready for field validation.

Work is underway to complete bench validation on real-time PCR assays for other OIE List A diseases such as RP, ASF, CBPP and LSD. The introduction of a foreign animal disease into the United States would have a great economic impact upon the agricultural system. Movement of animals and animal products would be interrupted until a clear epidemiological picture could be established. Therefore, it is essential that a rapid and accurate method of diagnosis is available. These assays will provide the agricultural industry and the US government with rapid, deployable assays that are sensitive and capable of detecting the presence of a foreign animal disease in the United States. Real-time PCR will play a critical role in determining the epidemiological pattern and management of a foreign animal disease outbreak within the United States.

IMPACT:

TECHNOLOGY TRANSFER:

The CSFV real-time RT-PCR assay was transferred from ARS to APHIS in April 2003. The FMDV real-time assay is scheduled to be transferred to APHIS within the first two weeks of September of 2003

Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus.

PUBLICATIONS:

Risatti, G.R., Callahan, J.D., Nelson, W.M., Borca, M.V. Rapid detection of classical swine fever virus by a portable real-time reverse transcriptase PCR assay. Journal of Clinical Microbiology. 2003 v. 41(1). p. 500-505.

Callahan JD, Brown F, Osorio FA, Sur JH, Kramer E, Long GW, Lubroth J, Ellis SJ, Shoulars KS, Gaffney KL, Rock DL, Nelson WM. J Am Vet Med Assoc. 2002 June 1;220(11):1636-42.

African Swine Fever Research

Project Title: Functional Pathogen Genomics: Virulence and Host Range Genes of swine Fever Virus
CRIS Number:: 1940-32000-036-00D
Scientists: Rock, D., Neilan, J., Zsak, L., Afonso, C., Lu, Z.
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PROJECT OBJECTIVES:
African swine fever (ASF) is a highly lethal hemorrhagic disease of domestic swine with mortality rates approaching 100%. The causative agent, African swine fever virus (ASFV), is a unique and genetically complex DNA virus. It is the sole member of a newly named virus family and the only known DNA arbovirus. Cycling of virus between soft ticks and the genus Ornithodoros and wild pig populations (wart hogs and bush pigs) in sub-Saharan Africa provides a natural reservoir of virus that poses a constant threat to domestic pig populations worldwide. There is no vaccine for ASF. Either a vaccine or other novel control methods is needed to reduce the threat posed by this highly significant viral disease.

Work here is focused on: 1) Identifying and characterizing viral genes responsible for virulence, viral latency and host range in both the swine and tick host; 2) Identifying host cell genes associated with host susceptibility and/or resistance;3) Developing vaccines and/or other novel approaches for disease control that exploit virulence and host range mechanisms; and 4) Developing genomic based methods to rapidly identify and phenotypically characterize viruses. Identification of viral virulence-associated genes will permit the rational design and genetic engineering of live attenuated viruses for use as vaccines. Additionally, an understanding of these genes and their functions may permit development of novel approaches for disease control that exploit aspects of viral host range.

OVERALL PROJECT ACCOMPLISHMENTS:
Characterization of the ASFV genome - The genome of a pathogenic African ASFV isolate, Malawi Lil-20/1, was sequenced and analyzed. Data provided primary structure of all encoded genes and overall genome organization. Sequence homologies with other known genes have had great predictive value for proposing and examining ASFV gene function and have led to the identification of significant swine virulence and host range genes. Additionally, these data have also facilitated the identification of putative immunogenic proteins - viral membrane and envelope proteins.

Identification of viral genes function in virulence and host range is difficult and very time consuming, especially when the genes are novel - lacking homology with other known genes. Putative ASFV virulence and host range genes were identified using a novel comparative genome approach. Analysis of complete genome sequences of seven pathogenic field isolates of ASFV identified several highly conserved genes in regions of the viral genome known to contain other pathobiologically significant genes. Impact - Comparative genomic approaches - facilitated by highly throughput DNA sequencing - may greatly simplify identification of novel genes with critical functions in the host. The strategy developed here for ASFV will have broad applicability for characterization of other threat agent pathogens.

Genetic engineering of the ASFV genome - Techniques were developed to genetically engineer pathogenic isolates of ASFV. These techniques which allow the addition or deletion of specific genes have permitted both genetic and reverse genetic research approaches for determining viral gene function. Recombinant ASFV gene-deletion mutants have been used to identify and characterize swine virulence and host range genes. This technology has proven critical for engineering live-attenuated ASFV vaccine viruses.

Identification and characterization of ASFV swine virulence and host range genes - Using genetic based strategies and ASFV gene-deletion mutants, a number of pathobiologically significant viral genes, many of which are novel, have been identified and characterized.

Virulence-associated genes, NL-S and UK were identified in pathogenic European ASFV isolates. Deletion of either of these genes from the viral genome resulted in complete attenuation of the virus in swine. A third distinct virulence determinant which includes multiple members of the multigene family 530 genes AVAD, was identi-fied in the left variable region of the genome. These genes which are required for viral virulence are novel, lacking similarity to any other known genes.

ASFV genes involved with the regulation of apoptosis in virus infected cells (bcl-2 and iap homologs) and genes with functions involving immune evasion in the swine host (CD2 and IkB homologs) were identified and characterized. CD2 and IkB genes had not been previously described in any virus. This is the first example of a pathogen using of a CD2-like molecule in immune evasion.

ASFV genes which affect macrophage host range were also identified and
characterized. The thymidine kinase gene (TK) and a second gene 9-GL are required for efficient replication of virus in infected cells; deletion of either gene resulted in a 99% to 99.9% reduction in viral growth and virulence attenuation.
9-GL is a novel gene whose function in virus replication is unknown. A novel macrophage growth-associated determinant which includes multiple members of the multigene family 360 genes was identified in the left variable region of the ASFV genome. These genes are novel lacking homology to other known genes.

ASFV and CSF infect and replicate in cells of the monocyte-macrophage lineage. Revealing molecular mechanisms involved in macrophage responses to infection is important to devise novel disease control strategies. A macrophage-specific swine cDNA microarray of 8029 clones was constructed and used to analyze the transcriptional profiles of swine macrophages infected with ASFV and gene deleted mutant viruses. Infection with virus caused a transcriptional response characterized by changes in the levels of expression of multiple macrophage genes. Up-regulated genes included novel and previously known genes, some of these involved in leukocyte differentiation, protein trafficking, endosome acidification and modulation of the antiviral response by nuclear factor kappa beta. Comparison of wild type and gene-deleted viruses revealed that ASFV genes from families 360 and 530 suppress early innate host responses induced by interferon alpha/beta. Mutant viruses containing deletion of 360 and 530 genes displayed enhanced expression of interferon induced early response genes and chemokines involved in the inflammatory response. Impact: These finding contribute to our understanding of swine resistance/susceptibility to viral infection and mechanisms of pathogenesis and may lead to early recognition of viral infection, predication of clinical outcome and identification of macrophage response genes associated with disease resistance.

A novel viral virulence determinant which includes multiple members of the MGF 530 genes has been identified in the left variable region of the ASFV genome using in vivo marker rescue. These genes will be an important target for engineering liveattenuated ASF vaccine viruses.

An understanding of pathogen-host interactions is critical for development of traditional and/or novel disease control strategies. The yeast two-hybrid system, used for the study of protein-protein interactions, was adapted specifically for investigating ASFV - swine macrophage interactions. ASFV infected swine macrophage cDNA libraries were constructed in a yeast activation domain GAL4 vector. Library Quality was assessed by nucleotide sequence and bio-informatic analysis. Two ASFV genes (5HL and 6FR) screened in this system, identified positive host cell interactor proteins. 5HL, an ASFV gene previously found to have a role in the modulation of apoptosis, baited host genes predicted to have roles in the regulation of programmed cell death pathways and cell cycle modulation. 6FR, a novel gene which has been implicated to have a critical role in ASFV infection, baited genes involved in host cell transcriptional regulation.

Overall, the identification of significant virulence and host range genes provides a rational basis for development of genetically engineered attenuated or nonreplicating (in the swine host) ASFV viruses for use as vaccines. Further, an understanding of how these novel genes function in the swine host will contribute to our overall understanding of viral virulence/host range, viral persistence and pathogen-vector-host interactions; information that may have broad impact on swine infectious disease in general.

Characterized latent infection with ASFV - Determined ASFV establishes a true latent infection in peripheral blood monocytes/macrophages, bone marrow and tonsil all recovered pigs, one in which viral replication does not occur. Knowledge of the existence of ASFV carrier animals is of critical importance for developing disease control programs in disease endemic regions.

Characterized ASFV-tick host interactions - Our identification of a large number of highly conserved ASFV genes with no apparent role in the swine host suggested these genes may be associated with aspects of infection in the tick host. Colonies of Ornithodoros ticks for use in investigating virus-tick interactions were established at PIADC from wild ticks collected in South Africa. Detailed pathogenesis studies of ASFV infection in the tick host revealed important aspects of this virus-host interaction. Recombinant ASF viruses containing deletions of putative tick host range genes were constructed and examined in the ticks. The viral TK gene has recently been shown to be necessary for viral replication in midgut epithelial cells. And, a second tick host range determinant has been mapped to the left variable region of the ASFV genome. Identification of ASF viral genes functioning in host range in Ornithodoros porcinus ticks. Viral genes necessary for infecting ticks were identified. Multigene family 360 genes are required for efficient virus replication and generalization of infection in O.porcinus ticks. Successful identification of tick host range genes will permit the design of live attenuated ASFV vaccine viruses that are incapable of infecting or being transmitted by ticks. The functional characterization of these genes, will also likely contribute to our understanding of pathogen-arthropod host interactions in general.

Functional genomics of cytoplasmic DNA viruses - A viral genomics approach was used to identify putative ASFV arthropod host range genes. Two cytoplasmic DNA viruses representing distinct virus families, Chlorella virus PBCV-1 (330 Kbp) and the Melanoplus Sanguinipes Entomopoxvirus (236 Kbp) and a Baculovirus pathogenic for Culex nigripalpus were completely sequenced and analysed. The Chlorella virus genome represents the largest viral genome sequenced to date. These data provided information on the primary

structure of all encoded genes, their organization, potential regulatory sequences and their evolutionary relationship to one another. In both viruses a number of novel and unexpected genes were identified. Sequence homologies with these viruses have led to the identification of ASFV genes with putative functions in virus-arthropod host interactions. This work has led to ASFV being placed into its own virus family, the Asfarviridae, and it has provided the genetic underpinning for all future research aimed at understanding the complex biology and evolution of large cytoplasmic DNA viruses.

Development of ASFV diagnostics and reagents - A PCR-based diagnostic assay for ASFV DNA, with a sensitivity equal to that of animal inoculation, was developed for use with blood and tissue samples. The assay is being used for research and diagnostic applications in the US, Spain, UK, South Africa, and China. Cloned genes, protein expression vectors, and both polyclonal and monoclonal antibody reagents have been developed and provided to research and diagnostic laboratories in the US, UK, Spain, South Africa, the Netherlands, and China.

The genomes of Fowlpox virus, Marek's disease virus, Turkey herpesvirus and Swine pox were completely sequenced and analyzed. An understanding of the genetic basis of viral virulence and host range will permit the engineering of novel vaccine viruses and expression vectors with enhanced efficacy and greater versatility.

The capripox viruses are responsible for highly significant foreign animal diseases (FAD) found in Africa and Central Asia and significantly they can be confused with a disease of sheep present in the United States caused by a parapoxvirus (orf virus). To improve diagnostic assays to distinguish between these viruses and to improve on existing vaccines for these diseases the genomes of lumpy skin disease-virus (151 kpb, 156 genes), sheeppox virus (146 kpb, 150 genes) and goatpox virus (151 kpb and 150 genes) were completely sequenced and analyzed. Virus specific gene targets were identified as were genes important for viral virulence and host range. Impact: Genomic sequences of these FAD viruses will allow for development of highly sensitive and specific diagnostic tests. Additionally, an understanding of the genetic basis of viral virulence and host range will permit the engineering of novel vaccine viruses and expression vectors with enhanced efficacy and greater versatility and will contribute novel concepts of pathogen-host interactions. This is fundamental information that will have broad impact on future strategies for controlling animal infectious diseases in general.

IMPACT:
The understanding of the genetic basis of ASFV virulence and host range generated here permit development of the first safe and efficacious ASF vaccine.

Information generated here on novel mechanisms of viral disease provide fundamental new insights on pathogen-host interactions and will have broad impact on future strategies for controlling animal infectious disease in general.

TECHNOLOGY TRANSFER:
Viruses, genomic sequences, cloned viral genes, protein expression vectors, and both polyclonal and monoclonal antibody reagents have been provided to research and diagnostic laboratories in the US, UK, Spain, the Netherlands, Denmark and China.

PUBLICATIONS:

Allende, R., Kutish, G.F., Laegreid, W.W., Lu, Z., Lewis, T.L., Rock, D.L., Friesen, J., Galeota, J.A., Doster, A.R. and Osorio, F.A. 2000. Mutations in the genome of porcine reproductive and respireatory syndrome virus responsible for the attenuation phenotype. Arch. Virol. 145:1149-1161.

Tulman, E.R., Afonso, C.L., Lu, Z., Zsak, L., Rock, D.L. and Kutish, G.F. 2000. The Genome of a Very Virulent Marek's Disease Virus. J. Virol. 74(17):7980-7988.

Afonso, C.L., Tulman, E.R., Lu, Z., Zsak, L., Rock, D.L. and Kutish, G.F. 2001. The Genome of Turkey Herpesvirus. J. Virol. 75(2):971-978.

Zsak, L., Lu, Z., Burrage, T.G., Neilan, J.G., Kutish, G.F., Moore, D.M. and Rock, D.L. 2001. African Swine Fever Virus Multigene Family 360 and 530 Genes are Novel Macrophage Host Range Determinants. J. Virol. 75(7):3066-3076.

Zsak, L., Sur, J-H., Burrage, T.G., Neilan, and Rock, D.L. 2001. African Swine Fever Virus Multigene Families 360 and 530 Genes Promote Infected Macrophage Survival. Scientific World Journal (1 Suppl 3):97.

Tulman, E.R. and Rock, D.L. 2001. Novel Virulence and Host Range Genes of African Swine Fever Virus. Current Opinion in Microbiology 4:456-461.

Zsak, L. and Neilan, J.G. 2002. Regulation of Apoptosis in African Swine Fever Virus-Infected Macrophages. Scientific World Journal 3(2):1186-11195.

Neilan, J.G., Zsak, L., Lu, Z., Kutish, G.F., Afonso, C.L. and Rock, D.L. 2002. A Novel Swine Virulence Determinant in the Left Variable Region of the African Swine Fever Virus Genome. J. Virol. 76(7):3095-3104.

Afonso, C.L., Piccone, M.E., Zaffuto, K.M., Neilan, J.G., Kutish, G.F., Lu, Z., Balinsky, C.A., Gibb, T.R., Bean, T.J., Zsak, L., and Rock, D.L. 2004. African Swine Fever Virus Multigene Family 360 and 530 Genes Affect Host Interferon Response. J. Virol. 78(4):1858-1864.

Burrage, T.G., Lu, Z., Neilan, Rock, D.L. and Zsak, L. 2004. African Swine Fever Virus Multigene Family 360 Genes Affect Virus Replication and Generalization of Infection in Ornithodoros Porcinus Ticks. J. Virol. 78(5):2445-2453.

African Swine Fever Research

Project Title: Identification and Characterization of African Swine Fever Virus Protective Antigens
CRIS Numbe: 1940-32000-037-00D
Scientists: Zsak, L., Rock, D., Neilan, J., Afonso, C., Vacant
Location: Plum Island Animal Disease Center, Office of the Center Director, African Swine Fever Research
Contact: Zsak, L., Phone: (631) 323-3023, Fax: (631) 323-3295
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PROJECT OBJECTIVES:
African swine fever (ASF) is a highly lethal hemorrhagic disease of domestic swine with mortality rates approaching 100%. The causative agent, African swine fever virus (ASFV), is a unique and genetically complex DNA virus. It is the sole member of a newly named virus family and the only known DNA arbovirus. Cycling of virus between soft ticks of the genus Ornithodoros and wild pig populations (wart hogs and bush pigs) in sub-Saharan Africa provides a natural reservoir of virus that poses a constant threat to domestic pig populations worldwide. There is no vaccine for ASF.

Either a vaccine or other novel control methods is needed to reduce the threat posed by this highly significant viral disease. The long term goal of the ASF research group at the Plum Island Animal Disease Center (PIADC) is development of an effective control strategy for this viral disease. This research project focuses on vaccination as a potential control strategy. Research involves defining the protective immune response to ASFV. Specifically to: 1) Evaluate genetically engineered ASFV with deletions of virulence/host range genes as vaccines for ASF; 2) Identify and characterize ASF viral proteins that induce a protective immune response in swine; 3) Define antigenic variability ASFV strains; 4) Define critical protective host immune responses; and 5) Develop new methods for effective vaccine delivery in swine. This research will allow evaluation of vaccination as a potential control measure for ASFV and will provide both the theoretical and practical basis for subsequent vaccine development.

OVERALL PROJECT ACCOMPLISHMENTS:
Our recent work in ASFV protective immunity has: 1) Clearly demonstrated the importance of the humoral immune response in protective immunity to ASFV infection; 2) Demonstrated the existence of neutralizing antibodies in the serum of recovered ASFV infected animals; 3) identified for the first time a specific viral protein (p72) involved in mediating virus neutralization and mapped the protein epitope responsible; 4) Demonstrated that ASFV neutralizing antibodies are not sufficient for antibody-mediated protection and that other antibody-mediated immune mechanisms are responsible for the protective effect; 5) Anti-ASFV antibodies mediating a novel anti-viral mechanism, monocyte infection-inhibition (M-II), were identified and shown to function in a strain-specific manner, suggesting a role for them in protective immunity; 6) Identified additional viral genes with possible significance to a protective immune response including those encoding, putative virion and infected cell membrane proteins, highly variable viral proteins and putative secreted viral virulence factors; 7) Demonstrated the feasibility of using genetically engineered live attenuated ASF viruses as vaccines. A live attenuated ASFV was constructed by deleting the 9GL gene (a gene required for efficient growth in swine macrophages). Pig infected (immunized) with this genetically engineered mutant virus were completely protected from lethal challenge with homologous and several heterologous virus strains indicating

that highly virulent African ASFV field isolates can be attenuated by gene deletion and that these viruses induce a protective immune response in swine; 8) Several virulent African strains of ASFV were attenuated by deleting virulence associated genes and then used in cross protection studies in pigs. Cross protection was observed for all South African viruses examined indicating that protection from heterologous viruses within a geographic region can be achieved. These data indicate that ASFV strain variation may not be as great as previously thought thus, vaccination with a single virus strain may be a viable option for controlling ASFV within a geographic region; and 9) Immune sera to different ASFV isolates were raised and tested in an in vitro monocyte infection-inhibition (M-II) assay where homologous and region-specific heterologous protective effects were observed. Significant correlation between the specificity of M-II assay and cross protection from heterologous viruses was observed. Findings will permit identification of viral proteins mediating M-II.

IMPACT:
Characterization of protective anti-viral host responses and viral antigens responsible for inducing them permit the development of a subunit vectored ASF vaccines that could be used in the U.S. in the event of a disease outbreak.

Insight into novel anti-viral host immune responses identified here will have broad impact on future strategies for controlling animal infectious disease in general.

TECHNOLOGY TRANSFER:
Viruses, genomic sequences, cloned viral genes, protein expression vectors, and both polyclonal and monoclonal antibody reagents have been provided to research and diagnostic laboratories in the US, UK, Spain, South Africa, the Netherlands, Denmark and China.

PUBLICATIONS:

Lewis, T., Zsak, L., Burrage, T.G., Lu, Z., Kutish, G.F., Neilan, J.G. and Rock, D.L. 2000. An African Swine Fever Virus ERV1-ALR Homologue, 9GL Affects Virion Maturation and Viral Growth in Macrophages and Viral Virulence in Swine. J. Virol. 74(3):1275-1285.

Neilan, J.G., Zsak, L., Lu, Z., Burrage, T.G., Kutish, G.F. and Rock, D.L. 2004. Neutralizing Antibodies to African Swine Fever Virus Proteins p30, p54 and p72 are not Sufficient for Antibody-Mediated Protection. Virology 319(2):337-342

Risatti, G.R., Borca, M.V., Zsak, L., Neilan, J.G., Lu, Z., Kutish, G.F. and Rock, D.L. 2004. Preclinical Diagnosis of African Swine Fever in Contact Exposed Swine by Portable Real-Time PCR Assay. J. Clin. Micro. (Submitted for Publication).

African Swine Fever Research

Project Title: Detection and Diagnosis of Hog Cholera (CSF)
CRIS Number:: 1940-32000-038-00D
Scientists: Vacant, Vacant
Location: Plum Island Animal Disease Center, Office of the Center Director, African Swine Fever Research

PROJECT OBJECTIVES:
Classical Swine Fever (CSF) is a highly infectious disease of swine. Morbidity and mortality rates in infected herds can be high. Infected sows can also transmit the virus to their offspring, creating asymptomatic carriers of the virus that shed virus in their feces, thereby contaminating the environment and posing risk to susceptible populations. As a foreign animal disease (FAD), an outbreak of CSF would significantly affect the U.S. pork industry and export markets. Improved CSF diagnostic tests and a marker live-attenuated CSF vaccine are needed to further reduce the risk posed by this FAD.

The goal of this research is directed at: 1) Developing molecular epidemiological capabilities for CSF; 2) Developing rapid field diagnostic tests for CSF; 3) Developing laboratory diagnostic tests that will differentiate field from marker vaccine strains of CSF; 4) Defining the pathogenesis of moderately virulent CSF viruses in pigs; 5) Identifying CSF virulence and host range determinants; and 6)
Developing a marker live attenuated CSF vaccine.

OVERALL PROJECT ACCOMPLISHMENTS:
Rapid identification and diagnosis of a FAD is critical for successfully controlling and eradicating the disease following introduction. Rapid field-based assays to detect CSFV are not yet available. A rapid real-time fluorogenic hydrolysis probe TaqMan assay was developed and evaluated in pigs experimentally infected with a moderately virulent CSF isolate, Haiti-96. The assay was highly sensitive and specific, detecting infected animals up to 5 days prior to the appearance of CSF clinical symptoms. Impact: This rapid, real-time RT-PCR assay for CSFV provides a new diagnostic tool that will redefine disease management and control strategies for this highly significant FAD. This is a new project initiated in FY 2001.

Engineering new markered live-attenuated CSF vaccines requires knowledge of viral virulence and host range determinants. Currently, little is known about CSFV in this regard. Insertional mutagenesis was used to define CSFV virulence and host range determinants. A CSFV genomic region associated with viral virulence was identified. Mutant viruses with changes in this region were: 1) completely attenuated in pigs; and 2) capable of inducing a protective immune response in pigs. Impact: The mapping of genetic determinants associated with viral virulence and host range represents the first step toward rational design of improved markered live-attenuated CSF vaccines.

IMPACT:
Rapid real-time PCR test for CSF provides new diagnostic tool that has redefined emergency disease management and control strategies for this high consequence disease.

Work here on identification and characterization of CSFV virulence and host range determinants permits rational design of improved markered live-attenuated CSF vaccines.

TECHNOLOGY TRANSFER:

Rapid detection method transferred to APHIS.

PUBLICATIONS:

Risatti, R.G., Callahan, J.D., Nelson, W.M., Boca, M.V. Rapid detection of Classical swine fever virus by a portable real-time reverse transcriptase PCR assay. Journal of Clinical Microbiology. 2003. v. 41(1). p. 500-505.

Risatti, G.R., Holinka, L.G., Lu, Z., Kutish, G.F., Callahan, J.D., Nelson, W.M., Brea Tio, E. and Borca, M.V. 2004. Diagnostic Evaluation of a Portable Real-Time Reverse Transcriptase PCR Assay for the Detection of Classical Swine Fever. J. Clin. Micro. (In Press).

African Swine Fever Research

Project Title: Advanced Animal Vaccines and Diagnostic Applications
CRIS Number:: 1940-32000-039-00D
Scientists: Rock, D., Neilan, J., Zsak, L., Lu, Z.
Location: Plum Island Animal Disease Center
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drock@cshore.com

PROJECT OBJECTIVES:
New disease control strategies are needed to reduce the threat posed by high consequence foreign animal diseases (FAD). Functional pathogen genomics offers an unprecedented opportunity for addressing this critical issue. High throughput pathogen genome sequencing together with detailed genome analysis will be used to provide improved tools for pathogen detection, disease diagnosis and epidemiological disease investigations, improved vaccines and therapeutics, and in all likelihood, completely novel disease control strategies with increased efficacy and utility. Specifically the goal of this research is to: 1) Develop methods for rapid strain identification and epidemiological investigations for key FAD agents; 2) Develop rapid field diagnostic tests for key FAD agents; 3) Develop improved vaccines for key FAD; and 4) Develop new methods on strategies for efficient and effective
disease control.

OVERALL PROJECT ACCOMPLISHMENTS:
Rapid field-based assays to detect foot and mouth disease virus (FMDV) are not yet available. A rapid RT-PCR assay was developed and evaluated in experimentally infected animals. The assay was both highly sensitive and specific and, it detected the virus before the animals showed signs of disease. Impact: A rapid (1 - 1 ½ hr) field-based diagnostic assay for FMDV provides a new and powerful tool that in all likelihood will revolutionize disease management and control strategies for this highly significant viral disease.

Canarypox virus (CPV) is a increasingly important mammalian vaccine vector; however, little information regarding the canarypox genome is currently available. The 360 kpb CPV genome was sequenced and compared to fowlpox virus, a related avipoxvirus of domestic birds. Analysis revealed 325 genes, including those conserved with fowlpox virus, other novel genes in expanded gene families, and virulence and host range genes unique to CPV. Impact: Knowledge of the entire coding capacity of CPV has identified specific genes which may affect unique aspects of CPV virulence and host range, allowing more directed studies to determine gene function in the host. Engineering of improved and more efficacious CPV vaccine vectors will result.

Poxviral infections in wild animal species are increasingly documented, indicating the presence of other, yet to be characterized poxviruses circulating in wild animal species. These can be confused with FAD viruses. Two deerpox viruses isolated in the western U.S. from free ranging mule deer were sequenced. Both virus genomes of 165 kbp shared 95% nucleotide identity. They differed from other known poxviruses both in central conserved regions and in terminal genomic regions where they contained a unique complement of virulence

and host range genes. Impact: Genomic information will permit design of improved diagnostic assays to distinguish between this virus and other exotic poxviruses associated with FAD of domestic animals.

IMPACT:
Rapid real-time PCR tests for high consequence foreign animal diseases developed here provides new diagnostic tools that have redefined emergency disease management and control strategies for these high significant diseases.

Pathogen comparative genomics has provided new tools for epidemiological and forensic investigations of foreign animal diseases and fundamental information that permit development of safer and more efficacious vaccines and vaccine vectors.

TECHNOLOGY TRANSFER:
Rapid FAD diagnostic tests for FMD, CSF and ASF have been developed. Evaluated and transferred to APHIS for use.

PUBLICATIONS:

Afonso, C.L., Tulman, E.R., Lu, Z., Zsak, L., Kutish, G.F. and Rock, D.L. 2000. The Genome of Fowlpox Virus. J. Virol. 74(8):3815-3831.

Tulman, E.R., Afonso, C.L., Lu, Z., Zsak, L., Kutish, G.F. and Rock, D.L. 2001. The Genome of Lumpy Skin Disease Virus. J. Virol. 75(15):7122-7130.

Afonso, C.L., Tulman, E.R., Lu, Balinsky, C.A., Moser, B.A., Becnel, J.J., Rock, D.L. and Kutish, G.F. 2001. Genome Sequence of a Baculovirus Pathogenic for Culex nigripalpus. J. Virol. 75(22):11157-11165.

Carrillo, C., Wigdorovitz, A., Trono, K., Dus Santos, M.J., Castanon, S., Sadir, A.m., Ordas, R. scribano, J.M. and Borca, M.V. 2001. Induction of a Virus-Specific Antibody Response to Foot-and-Mouth Disease Virus Using the Structural Protein VP1 Expressed in Transgenic Potato Plants. Viral Immunol. 14(1):49-57.

Trono, K.G., Perez-Filgueira, D.M., Duffy, S., Borca, M.V. and Carrillo, C. 2001. Seroprevalence of Bovine Leukemia Virus in Dairy a Cattle in Argentina: Comparison of Sensitivity and Specificity of Different Detection Methods. Vet. Microbiol. 83(3):235-248.

Gil, F., Brun, A., Wigdorovitz, A., Catala, R., Maertinez-Torrecuadrada, J.L., Casal, I., Salins, J., Borca, M.V. and Escribano, J.M. 2001. High-Yield Expression of a Viral Peptide Vaccine in Transgenic Plants. FEBS Lett. 488(1-2):13-17.

Afonso, C.L., Tulman, E.R., Lu, Z., Zsak, L., Osorio, F.A., Balinsky, C., Kutish, G.F. and Rock, D.L. 2002. The Genome of Swinepox Virus. J. Virol. 76(2):783-790.

Tulman, E.R., Afonso, C.L., Lu, Z., Zsak, L., Sur, J-H., Sandybaev, N.T., Kerembekova, U.Z., Zaitsev, V.L., Kutish, G.F. and Rock, D.L. 2002. The Genomes of Sheeppox and Goatpox Virus. J. Virology 76(12):6054-6061.

Afonso, C.L., Tulman, E.R., Lu, Z., Zsak, L., Sandybaev, N.T., Kerembekova, U.Z., Zaitsev, V.L., Kutish, G.F. and Rock, D.L. 2002. The Genome of Camelpox Virus. Virology 295(1):1-9.

Dus Santos, M.J., Wigdorovitz, A., Trono, K., Rioos, R.D., Franzone, P.M., Gil, F., Moreno, J., Carrillo, C., Escribano, J.M. and Borca, M.V. 2002. A Novel Methodology to Develop a Foot-and-Mouth Disease Virus Peptide-Based Vaccine in Transgenic Plants. Vaccine 20(7-8):1141-1147.

Ghosh, M.K., Borca, M.V. and Roy, P. 2002. Virus-Derived Tubular Structure Displaying Foreign Sequences on the Surface Elicit CD4+ Th Cell and Protective Humoral Responses. Virol. 302(2):383-392.

Delhon, G., Moraes, M.P., Lu, Z., Afonso, C.L., Flores, E.F., Weiblen, R., Kutish, G.F. and Rock, D.L. 2003. The Genome of Bovine Herpesvirus type 5. J. Virology 77(19):10339-10347.

Perez Filgueira, D.M., Zamorano, P.L., Dominguez, M.G., Taboga, O., Del Medico Zajac, M.P., Puntel, M., Romera, S.A., Morris, T.J., Borca, M.V. and Sadir, A.M. 2003. Bovine Herpes Virus gD Protein Produced in Plants Using a Recombinant Tobacco Mosaic Virus Vector Possesses Authentic Antigenicity. Vaccine 21(27-30):4201-4209.

Delhon, G., Tulman, E.R., Afonso, C.L., Lu, Z., de la Concha-Bermejillo, A., Lehmkuhl, H., Piccone, M.E., Kutish, G.F. and Rock, D.L. 2004. Genomes of the Parapoxvirus Orf Virus and Bovine Papular Stomatitis Virus. J. Virol. 78(1):168-177.

Tulman, E.R., Afonso, C.L., Lu, Z., Zsak, L., Kutish, G.F. and Rock, D.L. 2004. The Genome of Canarypox Virus. J. Virol. 78(1):353-366.

Lu, J., Tong, J., Huang, J., Afonso, C.L., Rock, D.L., Barany, F. and Weiguo, C. 2004. Ligation Properties as a New Class of NAD-Dependent DNA Ligase from Entomopoxvirus Melanoplus Sanguinipes. Biochimica et Biophysica Acta (In Press).

Kara, P.D., Afonso, C.L., Wallace, D.B., Kutish, G.F., Abolick, C., Lu, Z., Vreede, F.T., Vreede, F.T., Taljaard, L.C.F., Zsak, A., Viljoen, G.J., Rock, D.L. Comparative sequence analysis of the South African Vaccine Strain and Two Field Isolates of Lumpy Skin Disease Virus. Archives of Virology. 2003. Vol. 148(7). p. 1335-1356.

Lazo, A., Tassello, J., Jayarama, V., Ohagen, A., Gibaja, V., Kramer, E., Marmorato, A., Billia-Shaveet, D., Purrmal, A., Brown, F., Chapman, J. Broad-spectrum virus reduction in red cell concentrates using INACTINE TM PEN110 chemistry. Vox Sanguiniss. 2002. v. 83. p. 313-323.

Partidos, C.D., Biegnon, A-S., Brown, F., Kramer, E., Briand, J-P., Muller, S. Applying peptide antigens onto bare skin: induction of humoral and cellular immune responses and potential for vaccination. Journal Controlled Release. 2002. v. 85. p. 27-34.

Brown, F. The history of research in foot-and-mouth disease. Virus Research. 2003. v. 91. p. 3-7.

Fischer, D., Rood, D., Barrette, R.W., Zuwallack, A., Kramer, E., Brown, F., Silbart, L.K. Intranasal immunization of guinea pigs with an immunodominant foot-and-mouth disease virus peptide conjugate induces mucosal and humoral antibodies and protection against challenge. Journal of Virology. 2003. v. 77(12). p. 7486-7491.

Mather, T., Takeda, T., Tassello, J., Ohagen, A., Serebryanik, D., Kramer, E., Brown, F., Tesh, R., Alford, B., Chapman, J., Lazo, A. West nile virus in blood: stability, distribution, and susceptibility to PEN 110 inactivation. Transfusion. 2003. v. 43. p. 1029-1037.

African Swine Fever Research

Project Title: Advanced Veterinary Vaccines and Diagnostics
CRIS Number:: 1940-32000-039-04S
Scientists: Rock, D., Geary, S.
Location: Plum Island Animal Disease Center
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African Swine Fever Research
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PROJECT OBJECTIVES:
The US food animal economy has long been threatened by infectious diseases that could devastate the cattle, swine and poultry industries. Viral and bacterial infections affecting the airways and other mucosal tissues present the major risk to these economically important animal species. Pathogenic microbes are now, and will continue to be, the most significant cause of animal disease and economic loss. The easy pathogens have been dealt with, the difficult, intractable ones remain. New pathogens are continuously emerging and, in some cases, past disease control strategies (vaccines, antibiotics, other drugs and chemicals) are either losing effectiveness and/or public support for their use. Development and administration of new, more effective vaccines to counteract these pathogens will increase food safety, quality and production that will strengthen our markets at home and abroad.

OVERALL PROJECT ACCOMPLISHMENTS:
We have identified and characterized numerous virulence determinants in Mycoplasma gallisepticum and gained a greater understanding of the regulation of gene expression as well as posttranslational processing mechanisms of proteins in this pathogen. We have initiated studies aimed at determining the host response to this pathogen and assess how this contributes to the overall pathogenesis.

IMPACT:
Development of more effective vaccines for significant animal diseases will reduce losses associated with disease permitting increased food safety, quality and production and strengthen economic markets at home and abroad.

TECHNOLOGY TRANSFER:
"Use of a Live Attenuated Mycoplasma gallisepticum Strain as a Vaccine and Vector for the Protection of Chickens and Turkeys From Respiratory Disease" (Full patent filed 4/19/02).

PUBLICATIONS:

Papazisi,L., Silbart, L.K., Frasca Jr., S., Rood, D., Liao, X., Gladd, M. Javed, M.A. and Geary, S.J. A Modified Live Mycoplasma gallisepticum Vaccine to Protect Chickens From Respiratory Disease, Vaccine, 2002, 20(31-32):3709-3719.

Papazisi, L., Frasca, S. Jr., Gladd, M., Liao, X., Yogev, D., Geary, S.J. GapA and CrmA co-expression is essential for Mycoplasma gallisepticum cytadherence and virulence, Infect. Immunity, 2002, 70:6839-6845.

The complete genome sequence of Mycoplasma gallisepticum Strain R , GenBank Accession #: AE015450, October 17th, 2002.

Orenstein, S.M., Levisohn, S., Geary, S.J., Yogev, D. Cytadherence-Deficient Mutants of Mycoplasma gallisepticum Generated by Transposon Mutagenesis, Infect. Immunity, 2003, 71(7):3812-3820.

Papazisi, L., Gorton, T., Kutish, G., Markham, P., Browning, G., Nguyen, D., Swartzell, S., Madan, A., Mahairas, G. and Geary, S.J. "The complete genome sequence of the avian pathogen Mycoplasma gallisepticum strain Rlow". Microbiology,2003, 149(9):2307-2316

Zinckgraf, J.W. and Silbart, L.K. Modulating gene expression using DNA vaccines with different 3'-UTRs influences antibody titer, seroconversion rate and cytokine profile. Vaccine, 2003, 21(15):1640-1649.

Fisher, D., Rodd, D., Barrette, R.W., Zuwallack, A., Kramer, E., Brown, F. and Silbart, L.K. Intranasal immunization of guinea pigs with an immunodominant FMDV peptide conjugate induces mucosal and humoral antibodies and protection against challenge. J. Virology, 2003, 77(13):7486-7491.

African Swine Fever Research

Project Title: Program for Prevention of Animal Infections and Advanced Technologies for Vaccines and Diagnostics
CRIS Number:: 1940-32000-039-05S
Scientists: Rock, D., Estes, M.
Location: Plum Island Animal Disease Center
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PROJECT OBJECTIVES:
The infectious agents under study cause significant production losses to domestic food animals and include agents of foreign animal diseases that threaten a key segment of the agricultural economy. We are developing strategies for improved diagnostics and vaccine approaches to meet this challenge.

OVERALL PROJECT ACCOMPLISHMENTS:
We have begun to establish a comparative framework for genomic investigation of the Mycoplasma mycoides cluster of bovine and caprine pathogens that will be mined continually for potential diagnostic and therapeutic targets. This database will underpin new efforts to complete genomic sequences for the remaining species in this cluster, other bovine mycoplasma pathogens, and isolated strains of those species correlated with worldwide disease outbreaks. It is anticipated that the now completed genome sequence of M. capricolum subspecies capricolum Kid strain will drive the development of a genetic system for analysis of virulence traits for this group of organisms, leading to an accelerated understanding of their pathobiology and strategies to diagnose and treat these infectious diseases.

IMPACT:
Rapid diagnostic tests for high consequence foreign animal diseases will provide new diagnostic tools and will redefine emergency disease management and control strategies for these high significant diseases.

TECHNOLOGY TRANSFER:
The project has been underway for three years (since 3-1-00). Mycoplasmal genomic sequences will be deposited in public databases as they are annotated. Some delays in reaching these objectives occurred during the current period, reflecting the need to refine annotation methodology and to acquire the most recent comparative data. Prototype diagnostic applications are anticipated in the next two years. Vaccine material for tuberculosis is anticipated within the next two years. Adjuvant materials developed in this study show promise in tests in chimeric mice and can be applied to several vaccines under study within the consortium. Constraints may apply to the use of commercial materials obtained within the defined conditions of Material Transfer Agreements. New PRRSV infectious clones should be available within the next year and subsequent investigation is projected to provide potential vaccine reagents to commercial swine producers and scientists in the following several years. RNAi technology as an anti-parasitic sterilization therapy will be tested thoroughly in the coming year. Its application across the spectrum of animal parasitic diseases will be regulated by the pace by which parasite genomic sequence data become available.

PUBLICATIONS:

Austin, A., Haas, K.M., Jordan, K., Naugler, S. and Estes, D.M. Identification of a novel regulatory factor: Immunoglobulin A inducing protein. J. Immunol., 2003, 171:1336-1342.

Bajer, A., Jordan, K.R., Werling, D., Howard, C.J. and Estes, D.M. Ruminant dendritic cells derived from peripheral blood mononuclear cell precursors promote IgG1-restricted B cell responses in vitro. J. Leuk. Biol., 2003, 73:100-106.

Kleiboeker, S.B., Lee, S.M., Jones, C., and Estes, M.D. Lack of detectable BHV1, BVDV1 and BVDV2 shedding following immunization of calves with a modified-live vaccine. J. Am. Vet. Med. Assoc., 2003, 222:1399-403.

African Swine Fever Research

Project Title: Development of Rapid Real Time PCR-Based Assays for Selected OIE Class A Diseases
CRIS Number:: 1940-32000-041-00X
Scientists: Rock, D., Huxsoll, D., Rodriguez, L., McKenna, T.
Location: Plum Island Animal Disease Center
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PROJECT OBJECTIVES:
Although exotic livestock diseases, Foot-and-Mouth Disease Virus (FMDV), Classical Swine Fever (CSF) and Vesicular Stomatitis Virus (VSV) may enter the United States mainland by various means, the potential for terrorists, seeking vulnerable or soft targets in the US, to deliberately introduce foreign animal diseases has added a new dimension to livestock diseases as a threat to the national economy. Foreign animal diseases are a potential choice for terrorists in that many of the disease agents are easy to acquire, dissemination is not difficult, the diseases are readily transmissible, the livestock population is highly susceptible, and the economic impact of a major outbreak would be catastrophic.

To counter the new threat concerns the U.S. Department of Agriculture is developing a system of rapid real-time PCR assays for selected OIE class A animal pathogens to enable rapid detection and identification of diseased animals and effective management of disease outbreaks resulting from accidental or intentional introduction into the United States. This report serves to document the accomplishments of this project for fiscal year 2003.

OVERALL PROJECT ACCOMPLISHMENTS:
The major accomplishments over the life of this project include the design, development, optimization, and bench validation of a "dried-down" real-time RT-PCR assay for FMDV and CSFV. In addition, a real-time assay for VSV has been designed and optimized on wet-reagents and is now ready for production as a "dried-down" assay. All of the aforementioned assays have been or will be "dried-down" in a format specific for use in the SmartCycler (Cepheid, Sunnyvale, CA). The FMDV and CSFV assays are ready for field validation.

APHIS is finalizing agreements with foreign countries such as Argentina, Bolivia, Afghanistan, and Mexico to obtain samples from FMDV and CSFV outbreak areas in order to proceed with field validation of the real-time RT-PCR assays. Likewise, VSV field validation in negative animal cohorts will be initiated in the fall of 2003 through collaborative projects with Colorado State University and University of California-Davis (see annual reports for projects 1940-32000-040 03S and 1940-32000-041 03S) and through ongoing collaborations with ARS's Arthropod Borne Disease Research Laboratory (ABDRL). Field validation in positive cohorts for the VSV assay will be initiated in the fall of 2003 through collaborative agreements already in place with Mexico's Exotic Animal Disease Commission (5410-32000-012-01S and Costa Rica's School of Veterinary Medicine (5410-32000-012-02S). These collaborative agreements were established by ABDRL in FY03.

A training and proficiency test system has been put in place in order to have the real-time assays, once field validated, transferred to the NAHLN laboratories. BEI inactivated FMDV and CSFV isolates are currently being produced and tested both in vitro and in vivo. These inactivated viruses will be used in proficiency panels and as positive controls in the NAHLN laboratories. Once trained and tested, the NAHLN laboratory personnel will be capable of screening and helping diagnose the introduction of a foreign animal diseases into the United States. In the event of the outbreak, personnel trained to perform the real-time assays in the NAHLN laboratories would be available to assist the Foreign Animal Disease Diagnostic Laboratory at Plum Island in the diagnosis and management of additional cases.

Work is underway to complete bench validation on real-time PCR assays for other OIE List A diseases such as Rinderpest and African Swine Fever.

The introduction of a foreign animal disease into the United States would have a great economic impact upon the agricultural system. Movement of animals and animal products would be interrupted until a clear epidemiological picture could be established. Therefore, it is essential that a rapid and accurate method of diagnosis is available. These assays will provide the agricultural industry and the US government with rapid, deployable and sensitive tools to detect the presence of a foreign animal disease in the United States. Real-time PCR will play a critical role in determining the epidemiological pattern and management of a foreign animal disease outbreak within the United States.

IMPACT:
Rapid real-time PCR tests for high consequence foreign animal diseases developed here provides new diagnostic tools that have redefined emergency disease management and control strategies for these high significant diseases.

TECHNOLOGY TRANSFER:
The CSFV real-time RT-PCR assay was transferred from ARS to APHIS in April 2003. The FMDV real-time assay is scheduled to be transferred to APHIS within the first two weeks of September of 2003. APHIS will be training and proficiency testing NAHLN laboratory personnel on real-time RT-PCR using the Cepheid SmartCycler platform in the fall of FY'04. These two real-time assays will be deployed to the NAHLN laboratory network for negative field sample collection and diagnostic specificity testing in December (CSFV) and January/February (FMDV), respectively. Complete deployment of each assay to the end-user (NAHLN) is expected by March 2004 (CSFV) and July 1, 2004 (FMDV).

PUBLICATIONS:

Risatti, G.R., Callahan, J.D., Nelson, W.M., Borca, M.V. Rapid detection of classical swine fever virus by a portable real-time reverse transcriptase PCR assay. Journal of Clinical Microbiology. 2003. vol. 41(1). p. 500-505.

Callahan JD, Brown F, Osorio FA, Sur JH, Kramer E, Long GW, Lubroth J, Ellis SJ, Shoulars KS, Gaffney KL, Rock DL, Nelson WM. Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. J Am Vet Med Assoc. 2002 Jun 1;220(11):1636-42.

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PROJECTS

Dairy Processing and Products Research

- Biosecurity of Milk from the Farm to the Dairy Processing Plant

Dairy Processing and Products Research

Project Title: Biosecurity of Milk from the Farm to the Dairy Processing Plant
CRIS Number: 1935-41000-062-00D
Scientists: Tomasula, P., Rodriguez, L.
Location: Eastern Regional Research Center, Dairy Processing and Products Research
Contact: Tomasula, P., Phone: (215) 233-6703, x. 6703, Fax: (215) 233-6795
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PROJECT OBJECTIVES:
Foot and-mouth disease (FMD), caused by the foot-and-mouth disease virus (FMDV), is not a public health threat but is highly contagious to cloven-footed animals. The virus may be shed into milk up to 33 hrs before there are any apparent signs of the disease in dairy cows. Some of the literature on pasteurization of milk infected with FMDV reports that the virus survives in milk and milk products pasteurized according to current standards. Animals fed these products may then become infected with the virus.

The purpose of this project is to review past experiments and conduct additional relevant experiments to determine the thermal resistance of FMDV in milk and milk products and to ensure that current pasteurization standards for high temperature short time pasteurization, the method used in most processing plants, provides a safety margin for elimination of FMDV in milk and milk products. In addition, technologies that have been developed since the earlier research conducted in the '70s and that are currently available for dairy applications, will be applied to solve potential biosecurity problems at the farm, during transport of milk, and at the processing plant. This knowledge is needed for the development of science-based strategies for elimination of the virus in the event of an outbreak. The research and approaches are relevant to all pathogens that may cause animal safety or food safety concerns.

OVERALL PROJECT ACCOMPLISHMENTS:
This project was initiated in FY 2002, in collaboration with the Plum Island Animal Disease Center (PIADC) to support FMD research priorities. Both research locations are members of the FMD Dairy Research Working Group, an industry-driven group comprised of representatives from the dairy marketing cooperatives, milk processing groups, cheese processors, dairy farmers, representatives from the FDA – CFSAN and CVM and other government groups, and representatives from industry. The purpose of the FMD Dairy Research WG is to develop science-based guidelines that may be used by APHIS and local, state, and federal officials in the event of an outbreak to effect a coordinated response.

Methods Review: Examined the methodology that was used in experiments conducted in the '70s and 80's to determine the thermal resistance of foot-and-mouth disease in milk. This data was and still is used in the guidelines issued by APHIS and OIE for treatment of milk infected with FMDV. Concluded that much of the data was obtained using laboratory methods not in use today that do not simulate high temperature short time pasteurization. The data may over or under predict the thermal treatment delivered to milk in commercial pasteurization. Also, the data cannot be used to determine exact temperature-time relationships required to inactivate the FMD virus in milk as a function of log reduction of virus in milk. However, the data are useful to determine the ultimate effectiveness of a particular temperature in eliminating the virus in milk and past research has demonstrated that the difficulty in eliminating the virus in milk is most likely due to encapsulation of the virus by fat globule membrane or interaction with protein.

Review of guidelines: Current APHIS and OIE guidelines recommend that double pasteurization be used to eliminate virus in milk prior to feeding to animals. Determined from a literature review that these guidelines are not based on peer reviewed data but are an apparent extrapolation of data.

Pasteurization of infected milk: Experiments were conducted at PIADC, NY, to test the efficacy of HTST pasteurization for elimination of FMDV in milk using pilot plant scale equipment. This is preferable to use of a commercial unit because several thousand gallons of milk naturally infected with FMDV would be necessary for processing. In addition, milk containing at least 2log virus is required to measure pasteurization effectiveness, a level high enough for kinetic studies. Results from this study are also being interpreted in terms of on-farm destruction of milk.

At ERRC, developed methodology for determination of residence time distribution and fastest moving particle determination so that temperature-time relationships may be determined for pathogens in milk using smaller scale equipment and applied to commercial pasteurizers.

Milk tanker biosecurity : In preliminary work at ERRC, demonstrated the parameters for operation of the tanker at the farm and the processing plant, if existing tankers are retrofitted with equipment to secure the contents of the tanker.

IMPACT: Work performed to date has led to the strategies now being implemented by the dairy industry for improved biosecurity, in general, and for handling milk infected with FMDV at the farm, in transit, and at the processing plant. The strategies developed in this work are sufficiently general that their implementation will improve the security and safety of milk and dairy products against most pathogens.

TECHNOLOGY TRANSFER:
The results of the experiments are regularly discussed with dairy stakeholder groups and APHIS, USDA, and the FDA. The dairy stakeholder groups have shared the knowledge obtained in this study with their users.

PUBLICATIONS:

Tomasula, P.M. and R.P. Konstance. 2004. The survival of foot-and-mouth disease virus in raw and pasteurized milk and milk products. J. Dairy Sci. 87: 1115-1121.

Tomasula, P.M. and M.F. Kozempel. 2004. Flow characteristics of a pilot scale high temperature short time pasteurizer. Submitted to J. Dairy Science.

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PROJECTS

Periparturient Diseases of Cattle

- Molecular, Nutrient and Endocrine Basis for Metabolic Disease in the Transition Dairy Cow
- Identification of Factors Causing Immune Suppression at Parturition

Bacterial Diseases of Livestock

- Genomic and Immunological Analysis of Spirochetes that Infect Livestock
- Brucellosis: Vaccines, Immune Responses, Diagnostic Reagents
- Understanding Host-Pathogen Interactions for the Diagnosis and Control of Paratuberculosis (Johne's)
- Diagnosis and Control of Tuberculosis in Livestock and Wildlife

Virus and Prion Diseases of Livestock

- Emerging Viral Diseases of Swine
- Virus-Induced Respiratory and Reproductive Diseases of Swine
- Detection and Control of Bovine Viral Diarrhea Viruses
- Bovine Spongiform Encephalopathy and Other Transmissible Spongiform Encephalopathies

Respiratory Diseases of Livestock

- Respiratory Diseases of Poultry
- Control of Porcine Respiratory Diseases of Complex Etiology
- Haemophilus Parasuis Proteomics for Vaccine Design
- Controlling Losses from Respiratory Disease in Cattle, Sheep, and Goats
- Construction of Live Mucosal and Injectable Bacterial Vaccines Against Avian and Bovine Respiratory Diseases

Peripartorient Diseases of Cattle

Project Title: Molecular, Nutrient and Endocrine Basis for Metabolic Disease in the Transition Dairy Cow
CRIS Number:: 3625-32000-058-00D
Scientists: Reinhardt, T., Horst, R.
Location: Ames, IA, National Animal Disease Center, Periparturient Diseases of Cattle
Contact: Reinhardt, T., Phone: (515) 663-7540, Fax: (515) 663-7669, treinhar@nadc.ars.usda.gov

PROJECT OBJECTIVES:
Clinical hypocalcemia (Milk Fever) and subclinical hypocalcemia have an 8% and 75% incidence, respectively, in the periparturient dairy cow. Epidemiological studies have shown that metabolic diseases do not occur in isolation from each other, and hypocalcemia increases the incidence of other metabolic and infectious disorders in cows. It is clear that hypocalcemias are the initiators of a metabolic and infectious disease complex in the periparturient dairy cow. Hypocalcemia results in a 9-fold increase in ketosis, 6 times more dystosia, and a 4-fold increase in left displaced abomasum and retained placenta. In addition, the majority of infectious diseases (mastitis, salmonellosis, bovine viral diarrhea (BVD) and Johnes disease) that occur are associated with or increased by hypocalcemia. We are attempting to resolve this problem with an applied and basic research program focusing on understanding the diseases and improving methods we have developed to prevent hypocalcemia.

OVERALL PROJECT ACCOMPLISHMENTS:
Prior to our research, pre-calving diets high in calcium were thought to be the cause of milk fever. We proved this is not true in a seminal experiment with milk fever (hypocalcemia)-prone Jersey cows fed a high or low calcium diet, with either 1, 2, or 3% dietary potassium. Dietary calcium was found to have no effect on the development of milk fever or hypocalcemia. However the experiment did demonstrate that milk fever is primarily caused by high dietary potassium. Additional experiments suggest that high dietary potassium interferes with the ability of a key calcium regulating hormone – parathyroid hormone- to regulating the cow's blood calcium concentration .

We have also found the level of expression of a calcium-pumping gene in the mammary gland is a predictor of the metabolic disease complex associated with the stresses of parturition in dairy cows. We have developed reagents that can be used to study mammary gland calcium transport (during milk fever) and have potential as indicators of milk and milk protein production. We have contributed to the basic endocrinology/cell biology of the periparturient period in cows and model systems. We have identified factors predisposing cows to metabolic disease, such as age and diet, and we have established how these factors contribute to the development of the disease at the cellular level. For example, key hormone receptors involved in the control of blood calcium concentration were found to decline with age, the stresses of parturition, and the breed of cow. Diets to enhance the function of key hormone receptors have been developed and these are currently being applied for prevention of milk fever.

IMPACT:
These data have resulted in our development of new practical dietary supplements, which are now being used to lower the incidence of milk fever. As an outcome of our research, 50-60% of dairies now control potassium

content of diets fed to dry cows and 20-30% of dairies are now adding chloride supplements to adjust dietary cation-anion difference in their dry cow feeding programs.

TECHNOLOGY TRANSFER:
Our discovery that potassium is the cause of milk fever is now being included in every new veterinary medical textbook. The research was published in many of the major lay dairy publications in the U.S. Nearly all dairy nutritionists carefully determine dietary potassium when formulating rations for dairy cattle. Some are beginning to change agronomic practices designed to reduce potassium and increase chloride content of forages grown for dairy dry cows. Summaries of our recent experiments on the use of dietary hydrochloric acid as a means of counteracting high dietary potassium have also appeared in nearly every major lay dairy publication. The industry has been very quick to adopt the technology that has arisen from this project. Within months of our presentation of the work on hydrochloric acid at a scientific meeting, a company began marketing a feed supplement with hydrochloric acid to aid in prevention of milk fever. There are two companies (West Central Cooperative, Ralston, IA, and Nutritech Solutions, Ltd, Clearbrook, BC, Canada) now marketing products with HCl using formulas that are essentially our recipe. Technology transferred has also been scientific knowledge for use by other scientists and us. The knowledge should be reasonably durable but is subject to modification and change as the project's research moves forward. The probes and reagents developed should provide sound scientific tools required for further understanding of mammary function and milk production as well as tools for evaluation of practical metabolic disease prevention methods that will make it to the farmer.

T. Reinhardt

Invited to speak At the Stu Patten Wilderness Conference on Mammary Biology, on “Calcium Homeostasis in the Mammary Gland”, 2003

Invited to present a seminar at the Dept of Molecular Biology and Biochemistry ISU winter semester seminar series, on “Calcium transport in the Mammary Gland”, 2004

Invited to speak at the 18th Brunner Symposium at Michigan State Univ, “Calcium Pumps and Calcium Transport in the Mammary Gland”, 2004

R. Horst
Invited to lecture on “Role of Vitamin D in Calcium Homeostasis and its use in Milk Fever Prevention” at the Abildgaard Symposium on Hypocalcemia, Acidosis and Calcium Homeostasis, Copenhagen, Denmark, August 16-17, 2001.

Invited to lecture on “Troubleshooting Transition Rations for the Dairy Cow” at the 62nd Minnesota Nutrition Conference, Bloomington, MN, September 11-12, 2001.

Invited to lecture annual Wisconsin Agriculture Systems Association meeting on “Managing the Close UP Transition Cow” Wisconsin Dells, WI Jan 24-25 , 2002

Invited to lecture at the 17th annual Southwest Nutrition and Management Conference, on “Troubleshooting Transition Rations for the Dairy Cow” Feb 21-23, 2002, Pheonix, AZ

Invited to lecture to the University of Colorado Veterinary College on “Managing Hypocalcemia in the Periparturient Dairy Cow” March 19, 2002. Ft. Collins, CO

Invited to lecture at the annual Iowa Feed and Nutrition Seminar. Iowa State University, "Management of the transition dairy cow" April 23, 2002.

Invited to lecture at the Brown University DeLuca Symposium on Vitamin D on “Rat Cytochrome P450C24 (Cyp24) does not Metabolize 1,25-Dihydrovitamin D2 to Calcitroic Acid” Taos, NM, June 15-19, 2002.

Invited by Faculty of Veterinary Medicine, Warsaw Agricultural University to give a lecture at the Conference on “Prevention of Periparturient Diseases in Dairy Cows” September 4-5 2003, Warsaw, Poland.

Invited to co-author a review on “Vitamin D Metabolism” for Vitamin D 2nd edition edited by D. Feldman, F.H, Glorieux, J.W. Pike, 2004.

PUBLICATIONS:

Cheng YH, Goff JP, Sell JL, Dallorso ME, Gil S, Pawlak SE, Horst RL. Utilizing Solanum glaucophyllum alone or with phytase to improve phosphorus utilization in broilers. Poult Sci. 2004 Mar;83(3):406-13.

Foote MR, Horst RL, Huff-Lonergan EJ, Trenkle AH, Parrish FC Jr, Beitz DC. The use of vitamin D3 and its metabolites to improve beef tenderness. J Anim Sci. 2004 Jan;82(1):242-9.

Waldron MR, Nonnecke BJ, Nishida T, Horst RL, Overton TR. Effect of lipopolysaccharide infusion on serum macromineral and vitamin D concentrations in dairy cows. J. Dairy Sci. 2003 Nov;86(11):3440-6.

Waters WR, Palmer MV, Nonnecke BJ, Whipple DL, Horst RL. Mycobacterium bovis infection of vitamin D-deficient NOS2-/- mice. Microb Pathog. 2004 Jan;36(1):11-7.

Goff JP, Horst RL. Milk fever control in the United States. Acta Vet Scand Suppl. 2003;97:145-7. Review.

Goff JP, Horst RL. Role of acid-base physiology on the pathogenesis of parturient hypocalcaemia (milk fever)--the DCAD theory in principal and practice. Acta Vet Scand Suppl. 2003;97:51-6. Review.

Horst RL, Goff JP, Reinhardt TA. Role of vitamin D in calcium homeostasis and its use in prevention of bovine periparturient paresis. Acta Vet Scand Suppl. 2003;97:35-50. Review.

Waters WR, Nonnecke BJ, Foote MR, Maue AC, Rahner TE, Palmer MV, Whipple DL, Horst RL, Estes DM. Mycobacterium bovis bacille Calmette-Guerin vaccination of cattle: activation of bovine CD4+ and gamma delta TCR+ cells and modulation by 1,25-dihydroxyvitamin D3. Tuberculosis (Edinb). 2003;83(5):287-97.

Reinhardt TA, Horst RL, Waters WR. Characterization of Cos-7 cells overexpressing the rat secretory pathway Ca2+-ATPase. Am J Physiol Cell Physiol. 2004 Jan;286(1):C164-9. Epub 2003 Sep 10.

Nonnecke BJ, Waters WR, Foote MR, Horst RL, Fowler MA, Miller BL. In vitro effects of 1,25-dihydroxyvitamin D3 on interferon-gamma and tumor necrosis factor-alpha secretion by blood leukocytes from young and adult cattle vaccinated with Mycobacterium bovis BCG.
Int J Vitam Nutr Res. 2003 Jul;73(4):235-44.

Fujiwara I, Aravindan R, Horst RL, Drezner MK. Abnormal regulation of renal 25-hydroxyvitamin D-1alpha-hydroxylase activity in X-linked hypophosphatemia: a translational or post-translational defect. J Bone Miner Res. 2003 Mar;18(3):434-42.

Horst RL, Omdahl JA, Reddy S. Rat cytochrome P450C24 (CYP24) does not metabolize 1,25-dihydroxyvitamin D2 to calcitroic acid. J Cell Biochem. 2003 Feb 1;88(2):282-5.

Goff JP, Kimura K, Horst RL.Effect of mastectomy on milk fever, energy, and vitamins A, E, and beta-carotene status at parturition. J Dairy Sci. 2002 Jun;85(6):1427-36.

Kimura K, Goff JP, Kehrli ME Jr, Reinhardt TA. Decreased neutrophil function as a cause of retained placenta in dairy cattle. J Dairy Sci. 2002 Mar;85(3):544-50.

Zimmerman DR, Reinhardt TA, Kremer R, Beitz DC, Reddy GS, Horst RL. Calcitroic acid is a major catabolic metabolite in the metabolism of 1 alpha-dihydroxyvitamin D(2). Arch Biochem Biophys. 2001 Aug 1;392(1):14-22.

Reinhardt TA, Filoteo AG, Penniston JT, Horst RL. Ca(2+)-ATPase protein expression in mammary tissue. Am J Physiol Cell Physiol. 2000 Nov;279(5):C1595-602.

Langub MC, Reinhardt TA, Horst RL, Malluche HH, Koszewski NJ. Characterization of vitamin D receptor immunoreactivity in human bone cells. Bone. 2000 Sep;27(3):383-7.

Horst, R.L., Omdahl, J.L., Reddy, S. Rat cytochrome P450C24 (CYP24) does not metabolize 1,25-dihydroxyvitamin D2 to calcitroic acid. Journal of Cell Biochemistry. 2003. v. 88. p. 282-285.

Montgomery, J.L., Morrow Jr, K.J., Horst, R.L., Blanton, R.L., Galyean, M.L., Miller, M.F. Effect of vitamin D3 supplementation level on the postmortem tenderizatin of beef from steers. Journal of Animal Science. 2002. v. 80. p. 971-981.

Periparturient Diseases of Cattle

Project Title: Identification of Factors Causing Immune Suppression at Parturition
CRIS Number:: 3625-32000-068-00D
Scientists: Horst, R., Goff, J., Schmerr, M., Lippolis, J., Harp, J.
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PROJECT OBJECTIVES:

The major portion of health problems in dairy cattle occur at or around the time of calving; i.e., the periparturient period. These problems include mastitis, milk fever, retained placenta, and metabolic disorders such as ketosis and fatty liver. A common thread to many of these disorders may be diminished immune function. Our work will address mechanisms of immunity as they relate to two of the most economically important diseases of dairy cattle, mastitis and retained placenta. We will examine the effects of nutritional supplementation, milk production, or hormone treatments on periparturient immunosuppression in cows. We will test the actual activity of the immune system of the animals by intramammary challenge of the animals with common mastitis causing organisms such as the coliform and Streptococcus species. These studies will also provide information allowing us to investigate the relationship between immunological parameters we can measure in the lab and actual resistance to disease. These infection models also allow us to examine the molecular and cellular basis of the host-pathogen interaction in the bovine mammary gland to each bacterial infection. We are using proteomic analysis, as well as more traditional cytokine and leukocyte function assays to identify host proteins that are up or down-regulated during infection. We are working to determine whether bacterial protein expression differs inside the mammary gland from that observed when the bacteria are grown in culture media by comparing in vivo to in vitro grown bacterial protein expression. Ultimately antigens expressed in vivo, but not in vitro, could help define antigens necessary for incorporation into vaccines. We are also using the mammary gland infection model to compare the expression of lymphocyte cell surface receptors that regulate the migration and activation of immune cells in the mammary gland.

We have recently established an association between immune suppression and the retention of fetal membranes after calving (retained placenta). We continue to examine the immunologic parameters important in the etiology of retained placenta by identifying factors affecting neutrophil chemotaxis towards placental homogenate and attempting to modulate these interactions. A sensitive fluorescence immunoassay for IL8 using laser induced fluorescence on a capillary electrophoresis platform is under development. This assay will be able to measure IL8 at physiological levels. In addition, we are using genomic and proteomic analysis to compare protein profiles in placental tissues from cows with and without retained placenta to identify "disease-specific proteins" that may be critical to the success or failure of placental expulsion.

Completion of these objectives will result in an increased understanding of the mechanisms controlling two of the most economically important diseases in dairy cattle, mastitis and retained placenta. These findings will provide insight into rational measures for controlling these diseases.

OVERALL PROJECT ACCOMPLISHMENTS:

We demonstrated that the presence of the mammary gland affects the degree of immune suppression experienced by the cow during the periparturient period, most likely because milk production causes the cow to

experience prolonged periods of negative calcium and energy balance. These studies suggest that improved nutrition to address these two metabolic problems involving energy and calcium balance could have a positive impact on immune function and resistance to disease. Studies were then conducted to determine whether manipulation of the energy balance of cows at calving would have an impact on immune function. Cows outfitted with rumen cannulas were fed a balanced diet formulated to meet their energy needs. Control cows were allowed to undergo the usual decline in feed intake observed in most cows around the time of calving. However, treated cows were force-fed their diet through the rumen cannula so that energy intakes were not permitted to decline. This tactic succeeded in improving the energy balance of the cows but failed to prevent a decline in immune function in the cows. This research does not mean that energy balance is not important to the immune system but does suggest that improving energy balance alone will not restore full function to the immune system. The periparturient cow model we have developed permits assessment of the effects of a variety of nutritional factors on immune function around the time of calving.

The discovery of a role for the immune system in the etiology of retained placenta has redirected research focused on prevention of retained placenta into a whole new direction. Scientists now understand why the search for a link between the hormones associated with the act of parturition and retained placenta was so frustrating. Immune dysfunction is more likely at the heart of the retained placenta problem. We have discovered that cows developing retained placenta have lower levels of a cytokine, interleukin-8, in their blood than do cows that expel the placenta normally. Since interleukin-8 is considered the primary factor involved in neutrophil recruitment, a reason for the lack of production of this cytokine is our current focus.

IMPACT:
Demonstrating that all cows are immune suppressed for 1-2 weeks before and after calving has been instrumental in changing certain farm practices. For instance vaccination of cows on the day of calving was common because it was a convenient time to handle the cow. We are slowly convincing the industry that the cow will not respond to vaccinations administered at this time. We have clearly demonstrated that metabolic disease can worsen the degree of immune suppression experienced by the cow around the time of calving. Unfortunately we have not determined a way to fix damaged cows and our current focus is on understanding how to prevent the metabolic diseases from impacting the immune system through improved nutrition and management.

TECHNOLOGY TRANSFER:
Producers and veterinarians are excited about the implications of this research as it explains why vaccination protocols for periparturient cows often fail to provide protetion. Our work has clearly defined the major causes of the immune system shutdown. Producers are very familiar with the veterinary costs incurred during the first weeks of lactation in dairy cows from masitits and other infectious disease, and have been anxious to learn more about the factors that contribute to immune suppression in the newly calved cow. Scientists within this unit have been invited to many National and International meetings in the last two-three years to provide information on immune function in the periparturient cow and its implications to veterinarians, producers and allied industry personnel.

J. Goff

Invited to present a lecture on "Periparturient Immune Suppression and Nutrition" at the University of California-Davis Veterinary Continuing Education Program at the Tulare Farm Progress Show, Tulare, CA, February 10, 2001.

Invited to speak on "Milk Fever and Its Association with Metabolic Disease in Dairy Cows" at the Empire State Corn Days, Miner Institute, Chazy, NY, February 27, 2001.

Invited to speak on "Physiologic Factors to Consider in the Transition Dairy Cow" at the Western Dairy Management Conference, Las Vegas, NV, April 5, 2001.

Invited to speak at the Guelph University Continuing Education Conference for Dairy Practitioners on "New Research on Transition Cows", Guelph, Ontario, Canada May 30, 2001.

Invited to speak at the Feed Additives Update Conference for Dairy Nutritionists on "Adjusting DCAD and Minerals to Improve Transition Cow Health," East Lansing, MI, June 5, 2001.

Invited to speak on "The Role of Acid-base Physiology in Control of Hypocalcemia" and "Strategies for Prevention of Milk Fever" at the Abildegaard Veterinary Conference, Royal Veterinary College, Fredericksburg, Denmark, August 16, 2001.

Invited to present a seminar on "Mineral and DCAD Adjustments to Prevent Metabolic Disease" for the ZinPro-Balchem-Kemin joint Ohio Nutrition Conference, Akron, OH, August 28, 2001.

Invited to present a full-day pre-convention seminar on "Dry Cow Nutrition" at the Americasn Association of Bovine Practitioners Conference, VanCouver, British Columbia, Canada, September 12, 2001. (Unable to participate due to the tragedy of 09/11/01).

Invited to speak at the Nutritionist Conference for Dairy Consultants on "Dry Cow Physiology and Nutrition," Albuquerque, NM, September 26, 2001.

Invited to speak at the Idaho Feed Industry Consortium of Dairy Nutritionists on "Dry Cow Nutrition," Boise, ID, September 28, 2001.

Invited to present a Dairy Science departmental seminar on "Physiology of the Transitin Cow" at Penn State University, State College, PA, November 16, 2001.

Invited to speak at the Colorado Nutrition Conference on "Mineral Management of the Dry Cow, Including Oral Drenching," Greeley, CO, January 29, 2002.

Invited to give a lecture in the Plenary Session of the National Mastitis Council meeting on "Mechanisms by Which Metabolic Diseases Predispose Cows to Infectious Disease," Orlando, FL, February 5, 2002.

Presented an invited paper on "Management and feeding of the transition cow " at the Central Wisconsin Nutrition Meeting, July 23, 2003 in Eau Claire, WI. (75 dairy nutritionists)

Taught section of a course on dairy herd management and troubleshooting to senior veterinary students at the Iowa State University Veterinary College from August 11-15 2003. (30 veterinary students)

Taught 2 day seminar on nutrition and management of the transition cow at the American Association of Bovine Practitioners Meeting in Columbus Ohio on September 16 and 17, 2003.

Presented a series of dairy extension talks on "Metabolic disease control and mastitis resistance" to dairy producers and veterinarians in Valdosta, Ga September 17, 2003,; Chiefland, FL on September 18, 2003; and Okeechobee, FL on September 19, 2003.

Presented a paper on "Effect of the presence of the mammary gland on periparturient immune function" at the USDA-ARS Immunology Conference in Bethesda MD on December 2, 2003.

Presented talks on "Macromineral nutrition" and "Factors affecting trace mineral requirements of cattle" at the Shur-gain Nutrition Conference in Stratford, Ontario, Canada on December 4, 2003.

Served on Committee on Animal Nutrition for the National Research Council of the National Academies of Science.

Serving on the subcommittee of the Board on Agriculture of the National Academy of Science to revise the book "Mineral Tolerances of Domestic Animals".

Section Editor of the Digestive Physiology and Metabolism portion of the revised edtition of the book "Duke's Physiology of Domestic Animals" which is a primary textbook for veterinary students.

M. Schmerr

Presented paper "Isolation of the Cytokine IL8 from Bovine Pla sma" HPCE2003 in San Diego, CA January 2003. Mary Jo Schmerr and Kayoko Kimura

Presented paper "Analysis of a Fluorescence Immunoassay for the Cytokine IL-8 by Capillary Electrophoresis" at HPCE2004 in Salzburg, Austria February 2004
Mary Jo Schmerr and Kayoko Kimura

J. Harp

Chaired Immunology Section at 2002 (St. Louis) and 2003 (Chicago) Annual Meetings of Conference of Research Workers in Animal Diseases.

PUBLICATIONS:

Cheng YH, Goff JP, Sell JL, Dallorso ME, Gil S, Pawlak SE, Horst RL. Utilizing Solanum glaucophyllum alone or with phytase to improve phosphorus utilization in broilers.
Poult Sci. 2004 Mar;83(3):406-13.

Harp, J.A., Stabel, J.R., Pesch, B.A. and Goff, J.P. Expression of adhesion molecules on milk and blood leukocytes from periparturient dairy cattle with Johne's disease. Vet. Immunol. Immunopathol. 98:69-76. 2004.

Stabel JR, Goff JP, Kimura K. Effects of supplemental energy on metabolic and immune measurements in periparturient dairy cows with Johne's disease.
J Dairy Sci. 2003 Nov;86(11):3527-35.

Goff JP, Horst RL. Milk fever control in the United States. Acta Vet Scand Suppl. 2003;97:145-7. Review.

Harp JA. Cryptosporidium and host resistance: historical perspective and some novel approaches. Anim Health Res Rev. 2003 Jun;4(1):53-62. Review.

Chen W, Harp JA, Harmsen AG. Cryptosporidium parvum infection in gene-targeted B cell-deficient mice. J Parasitol. 2003 Apr;89(2):391-3.

Goff JP, Horst RL. Role of acid-base physiology on the pathogenesis of parturient hypocalcaemia (milk fever)--the DCAD theory in principal and practice.
Acta Vet Scand Suppl. 2003;97:51-6. Review.

Horst RL, Goff JP, Reinhardt TA. Role of vitamin D in calcium homeostasis and its use in prevention of bovine periparturient paresis. Acta Vet Scand Suppl. 2003;97:35-50. Review.

Melendez P, Donovan GA, Risco CA, Littell R, Goff JP. Effect of calcium-energy supplements on calving-related disorders, fertility and milk yield during the transition period in cows fed anionic diets.
Theriogenology. 2003 Sep 15;60(5):843-54.

Nonnecke BJ, Kimura K, Goff JP, Kehrli ME Jr. Effects of the mammary gland on functional capacities of blood mononuclear leukocyte populations from periparturient cows.
J Dairy Sci. 2003 Jul;86(7):2359-68.

Trott DJ, Moeller MR, Zuerner RL, Goff JP, Waters WR, Alt DP, Walker RL, Wannemuehler MJ. Characterization of Treponema phagedenis-like spirochetes isolated from papillomatous digital dermatitis lesions in dairy cattle. J Clin Microbiol. 2003 Jun;41(6):2522-9.

Harp, J.A. Parasitic infections of the gastrointestinal tract. Curr. Opin. Gastroenterol. 19:31-36. 2003.

Kimura K, Goff JP, Kehrli ME Jr, Harp JA, Nonnecke BJ. Effects of mastectomy on composition of peripheral blood mononuclear cell populations in periparturient dairy cows.
J Dairy Sci. 2002 Jun;85(6):1437-44.

Goff JP, Kimura K, Horst RL. Effect of mastectomy on milk fever, energy, and vitamins A, E, and beta-carotene status at parturition. J Dairy Sci. 2002 Jun;85(6):1427-36.

Kimura K, Goff JP, Kehrli ME Jr, Reinhardt TA. Decreased neutrophil function as a cause of retained placenta in dairy cattle. J Dairy Sci. 2002 Mar;85(3):544-50.

El-Samad H, Goff JP, Khammash M. Calcium homeostasis and parturient hypocalcemia: an integral feedback perspective. J Theor Biol. 2002 Jan 7;214(1):17-29.

Lippolis JD, White FM, Marto JA, Luckey CJ, Bullock TN, Shabanowitz J, Hunt DF, Engelhard VH. Analysis of MHC class II antigen processing by quantitation of peptides that constitute nested sets. J Immunol. 2002 Nov 1;169(9):5089-97.

Tang M, Harp JA, Wesley RD. Recombinant adenovirus encoding the HA gene from swine H3N2 influenza virus partially protects mice from challenge with heterologous virus: A/HK/1/68 (H3N2). Arch Virol. 2002 Nov;147(11):2125-41.

Sonea IM, Palmer MV, Akili D, Harp JA. Treatment with neurokinin-1 receptor antagonist reduces severity of inflammatory bowel disease induced by Cryptosporidium parvum.
Clin Diagn Lab Immunol. 2002 Mar;9(2):333-40.

Waters WR, Nonnecke BJ, Rahner TE, Palmer MV, Whipple DL, Horst RL.
Modulation of Mycobacterium bovis-specific responses of bovine peripheral blood mononuclear cells by 1,25-dihydroxyvitamin D(3). Clin Diagn Lab Immunol. 2001 Nov;8(6):1204-12.

Sunita Rao D, Balkundi D, Uskokovic MR, Tserng K, Clark JW, Horst RL, Satyanarayana Reddy G. Double bond in the side chain of 1alpha,25-dihydroxy-22-ene-vitamin D(3) is reduced during its metabolism: studies in chronic myeloid leukemia (RWLeu-4) cells and rat kidney.
J Steroid Biochem Mol Biol. 2001 Aug;78(2):167-76.

Luckey CJ, Marto JA, Partridge M, Hall E, White FM, Lippolis JD, Shabanowitz J, Hunt DF, Engelhard VH. Differences in the expression of human class I MHC alleles and their associated peptides in the presence of proteasome inhibitors. J Immunol. 2001 Aug 1;167(3):1212-21.

Schell TD, Lippolis JD, Tevethia SS. Cytotoxic T lymphocytes from HLA-A2.1 transgenic mice define a potential human epitope from simian virus 40 large T antigen.
Cancer Res. 2001 Feb 1;61(3):873-9.

Chen, W., Chadwick, V.S., Tie, A., and Harp, J.A. Cryptosporidium parvum in intestinal mucosal biopsies from patients with inflammatory bowel disease. Am. J. Gastroenterol. 96:3463-3464. 2001.

Purdy, C.W., Straus, D.C., Harp, J.A., Mock, R., and Popham, T.W. Microbial pathogen survival in a high plains feedyard playa. Texas J. Sci. 53:247-266. 2001

Kehrli ME Jr, Harp JA. Immunity in the mammary gland. Vet Clin North Am Food Anim Pract. 2001 Nov;17(3):495-516, vi. Review.

Heidari M, Harp JA, Kehrli ME Jr. Expression, purification, and in vitro biological activities of recombinant bovine granulocyte-colony stimulating factor. Vet Immunol Immunopathol. 2001 Aug 30;81(1-2):45-57.

Moore D, Waters WR, Wannemuehler MJ, Harp JA. Treatment with agmatine inhibits Cryptosporidium parvum infection in infant mice. J Parasitol. 2001 Feb;87(1):211-3.

Nepom GT, Lippolis JD, White FM, Masewicz S, Marto JA, Herman A, Luckey CJ, Falk B, Shabanowitz J, Hunt DF, Engelhard VH, Nepom BS. Identification and modulation of a naturally processed T cell epitope from the diabetes-associated autoantigen human glutamic acid decarboxylase 65 (hGAD65). Proc Natl Acad Sci U S A. 2001 Feb 13;98(4):1763-8.

Lippolis JD, Denis-Mize KS, Brinckerhoff LH, Slingluff CL Jr, Galloway DR, Engelhard VH. Pseudomonas exotoxin-mediated delivery of exogenous antigens to MHC class I and class II processing pathways. Cell Immunol. 2000 Aug 1;203(2):75-83.

Pelan-Mattocks LS, Kehrli ME Jr, Casey TA, Goff JP. Fecal shedding of coliform bacteria during the periparturient period in dairy cows. Am J Vet Res. 2000 Dec;61(12):1636-8.

Hagemoser WA, Goff JP, Sanderson TP, Haynes JS. Osteopenic disease in growing pigs: diagnostic methods using serum and urine calcium and phosphorus values, parathoromone assay, and bone analysis. J Vet Diagn Invest. 2000 Nov;12(6):525-34.

Goff JP. Pathophysiology of calcium and phosphorus disorders. Vet Clin North Am Food Anim Pract. 2000 Jul;16(2):319-37, vii. Review.

Moore SJ, VandeHaar MJ, Sharma BK, Pilbeam TE, Beede DK, Bucholtz HF, Liesman JS, Horst RL, Goff JP. Effects of altering dietary cation-anion difference on calcium and energy metabolism in peripartum cows. J Dairy Sci. 2000 Sep;83(9):2095-104.

Akili D, Harp JA. A factor derived from adult rat and cow small intestine reduces Cryptosporidium parvum infection in infant rats. J Parasitol. 2000 Oct;86(5):979-82.
Waters WR, Palmer MV, Wannemuehler MJ, Sacco RE, Harp JA. B cells are required for the induction of intestinal inflammatory lesions in TCRalpha-deficient mice persistently infected with Cryptosporidium parvum. J Parasitol. 2000 Oct;86(5):1073-7.

Waters WR, Frydman B, Marton LJ, Valasinas A, Reddy VK, Harp JA, Wannemuehler MJ, Yarlett N. [(1)N,(12)N]Bis(Ethyl)-cis-6,7-dehydrospermine: a new drug for treatment and prevention of Cryptosporidium parvum infection of mice deficient in T-cell receptor alpha.
Antimicrob Agents Chemother. 2000 Oct;44(10):2891-4.

Jackman R, Schmerr MJ. Analysis of the performance of antibody capture methods using fluorescent peptides with capillary zone electrophoresis with laser-induced fluorescence.
Electrophoresis. 2003 Mar;24(5):892-6.

Hamir AN, Miller JM, Schmerr MJ, Stack MJ, Chaplin MJ, Cutlip RC. Diagnosis of preclinical and subclinical scrapie in a naturally infected sheep flock utilizing currently available postmortem diagnostic techniques. J Vet Diagn Invest. 2001 Mar;13(2):152-4.

Bacterial Diseases of Livestock

Project Title: Genomic and Immunological Analysis of Spirochetes that Infect Livestock
CRIS Number: 3625-32000-060-00D
Scientists: Zuerner, R., Alt, D., Whipple, D. Stanton, T. Vacant
Location: Ames, IA, National Animal Disease Center, Bacterial Diseases of Livestock
Contact: Zuerner, R., Phone: (515) 663-7392, Fax: (515) 663-7458
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PROJECT OBJECTIVES:

Spirochetes are bacteria that cause a variety of diseases in animals and humans. Our project studies spirochete diseases of livestock caused by *Leptospira*, *Brachyspira*, and *Treponema*. Leptospirosis, caused by *Leptospira*, is a common cause of reproductive failure (abortion, stillbirth, and production of weak offspring) in livestock. Diagnosis or isolation of these bacteria in culture is difficult, but essential for controlling the spread of disease. Leptospirosis can be resolved through the use of antibiotics, and vaccines provide some protection. However, the effectiveness of vaccination varies among animal species. Furthermore, *Leptospira* strains are diverse, and vaccination produces specific immunity with little cross protection against different strains. The Spirochete Diseases Research Project, Genomic and Immunological Analysis of Spirochetes That Infect Livestock, at the USDA-ARS National Animal Disease Center (NADC), Ames, IA, is developing novel and sensitive methods for diagnosis of spirochetes in livestock, conducting genetic and pathogenesis studies to determine how these bacteria cause disease, and identifying new targets for vaccine development. *Brachyspira* are intestinal spirochetes that cause swine dysentery and porcine intestinal spirochetosis. These diseases can cause significant losses in swine production. Porcine intestinal spirochetosis is difficult to diagnose. Therefore, we are developing tools for serological diagnosis of infection. *Brachyspira* are also the only spirochetes with a known mechanism of genetic exchange. The process by which these bacteria exchange DNA is unusual, and we are characterizing the process to determine its usefulness for genetic manipulation of spirochetes. Members of the genus *Treponema* are associated with papillomatous digital dermatitis (PDD), the primary cause of lameness in dairy cattle. We are characterizing the bacteria associated with this disease to develop diagnostic probes and study how these bacteria contribute to the disease.

OVERALL PROJECT ACCOMPLISHMENTS:

Leptospiral vaccines for cattle have been available for years, but field data suggested that effectiveness for protection against certain types of bovine leptospirosis was not adequate. A new vaccine that was commercially available in Australia, New Zealand, Ireland and the United Kingdom was evaluated to determine if it would protect cattle challenged with a United States strain of *Leptospira*. The vaccine prevented colonization and shedding of the organism and protected animals against infection. This was in contrast to poor protection that resulted when cattle were vaccinated with other commercially available leptospiral vaccines.

Immune responses stimulated by the effective vaccine were characterized to determine why this vaccine was effective when others were not. Results of this research demonstrated that a potent cell mediated response was important for protection. This information is important for understanding how to design other effective vaccines against leptospirosis and other diseases.

The composition of the *Leptospira* outer membrane was characterized with the discovery of several major surface exposed proteins. These proteins are the subjects of studies being done in conjunction with academic collaborators aimed at developing subunit vaccines for leptospirosis. The major outer membrane protein, LipL32, was characterized and is well conserved among pathogenic leptospira species. This protein is highly antigenic and is potentially useful for both diagnosis and vaccine development.

Cattle infected with *Leptospira* were treated with streptomycin until its withdrawal from the U.S. market. Alternative antibiotic treatments were tested for efficacious treatment of bovine leptospirosis. This work provides essential benchmark data that can be used in negotiating export-import restrictions designed to limit the spread of leptospirosis.

The genome of *Leptospira borgpetersenii* serovar *hardjo*, the primary cause of bovine leptospirosis, is being sequenced. Analysis of genome sequence data led to identification of a surface exposed protein conserved among pathogenic spirochetes. Conserved and variable regions of other outer membrane proteins were identified and characterized. Combined, these data will form the basis for subunit vaccine development for leptospirosis.

Bacteria were isolated from PDD lesions and characterized. These *Treponema phagedenis*-like bacteria are similar to bacteria detected in PDD lesions from cattle around the world, and their isolation in pure culture is a key step in identifying the cause of this disease and developing strategies to control the development of PDD. Cattle with PDD developed antibody to these bacteria and also developed a cellular immune response to bacterial antigens. Using a technique called Serial Analysis of Gene Expression (SAGE), the global response of bovine macrophages to a *Treponema phagedenis*-like bacterium isolated from a PDD lesion was characterized. This study is the first description of global host response to any spirochete, and therefore is breaking new ground in the field. The results of these studies are providing key information on how these spirochete bacteria affect the host, and thereby establish infection.

Methods were developed to isolate the outer membrane of *Brachyspira*, leading to identification of outer membrane proteins. Outer membrane proteins are ideal targets for vaccine development, and may be useful for sensitive diagnostic assays. These studies established an essential framework for analysis of porcine intestinal spirochetosis.

IMPACT:
Understanding host immune responses stimulated by effective vaccines will lead to development of improved vaccines for leptospirosis as well as other diseases. Identification and characterization of specific proteins and genes of *Leptospira* could lead to development of efficacious subunit vaccines. Improved vaccines to prevent disease will result in decreased economic losses to the livestock industry.
Characterizing the organisms that cause PPD is important for developing tools to treat and prevent the disease, which is a leading cause of lameness in dairy cattle and results in significant economic loss.

Determining the whole genomic sequence of *Leptospira borgpetersenii* serovar *hardjo* will lead to identification of genes and proteins that are associated with virulence of the organism and clues to how the organism causes disease. This information can be used to develop improved diagnostic tests, vaccines, and other intervention strategies.

TECHNOLOGY TRANSFER:
Methods and reagents were provided to diagnostic laboratories to aid in diagnosis of leptospirosis in livestock, and to assist in isolation of these bacteria in pure culture. *Leptospira* challenge strains for testing the efficacy of vaccines were provided to biologics firms, academic laboratories, and to the Center for Veterinary Biologics. ARS scientists have assisted in evaluation of leptospiral vaccines in collaboration with biologics firms.

PUBLICATIONS:

Haake DA, Suchard MA, Kelley MM, Dundoo M, Alt DP, Zuerner RL. Molecular Evolution and Mosaicism of Leptospiral Outer Membrane Proteins Involves Horizontal DNA Transfer. J Bacteriol. 2004; 186(9):2818-2828.

Trott DJ, Alt DP, Zuerner RL, Bulach DM, Wannemuehler MJ, Stasko J, Townsend
KM, Stanton TB. Identification and cloning of the gene encoding BmpC: an outer-membrane lipoprotein associated with *Brachyspira pilosicoli* membrane vesicles. Microbiology. 2004;150(Pt 4):1041-1053.

Stanton TB, Thompson MG, Humphrey SB, Zuerner RL. Detection of bacteriophage VSH-1 svp38 gene in *Brachyspira* spirochetes. FEMS Microbiol Lett. 2003;224(2):225-9.

Trott DJ, Moeller MR, Zuerner RL, Goff JP, Waters WR, Alt DP, Walker RL, Wannemuehler MJ. Characterization of *Treponema phagedenis*-like spirochetes isolated from papillomatous digital dermatitis lesions in dairy cattle. J Clin Microbiol. 2003; 41(6):2522-9.

Cullen PA, Haake DA, Bulach DM, Zuerner RL, Adler B. LipL21 is a novel surface-exposed lipoprotein of pathogenic *Leptospira* species. Infect Immun. 2003;71(5):2414-21.

Zuerner RL, Huang WM. Analysis of a *Leptospira interrogans* locus containing DNA replication genes and a new IS, IS1502. FEMS Microbiol Lett. 2002; 215(2):175-82.

Naiman BM, Blumerman S, Alt D, Bolin CA, Brown R, Zuerner R, Baldwin CL. Evaluation of type 1 immune response in naive and vaccinated animals following challenge with *Leptospira borgpetersenii* serovar *Hardjo*: involvement of WC1(+) gammadelta and CD4 T cells. Infect Immun. 2002; 70(11):6147-57.

Trott DJ, Alt DP, Zuerner RL, Wannemuehler MJ, Stanton TB. The search for *Brachyspira* outer membrane proteins that interact with the host. Anim Health Res Rev. 2001; 2(1):19-30.

Naiman BM, Alt D, Bolin CA, Zuerner R, Baldwin CL. Protective killed *Leptospira borgpetersenii* vaccine induces potent Th1 immunity comprising responses by CD4 and gammadelta T lymphocytes. Infect Immun. 2001; 69(12):7550-8.

Alt DP, Zuerner RL, Bolin CA. Evaluation of antibiotics for treatment of cattle infected with *Leptospira borgpetersenii* serovar hardjo. J Am Vet Med Assoc. 2001;219(5):636-9.

Brenot A, Trott D, Saint Girons I, Zuerner R. Penicillin-binding proteins in *Leptospira interrogans*. Antimicrob Agents Chemother. 2001; 45(3):870-7.

Hsu T, Hutto DL, Minion FC, Zuerner RL, Wannemuehler MJ. Cloning of a beta-hemolysin gene of *Brachyspira (Serpulina) hyodysenteriae* and its expression in *Escherichia coli*. Infect Immun. 2001; 69(2):706-11.

Bolin CA, Alt DP. Use of a monovalent leptospiral vaccine to prevent renal colonization and urinary shedding in cattle exposed to *Leptospira borgpetersenii* serovar hardjo. Am J Vet Res. 2001; 62(7):995-1000.

Wagenaar J, Zuerner RL, Alt D, Bolin CA. Comparison of polymerase chain reaction assays with bacteriologic culture, immunofluorescence, and nucleic acid hybridization for detection of *Leptospira borgpetersenii* serovar hardjo in urine of cattle. Am J Vet Res. 2000 Mar;61(3):316-20.

Bacterial Diseases of Livestock

Project Title: Brucellosis: Vaccines, Immune Responses, Diagnostic Reagents
CRIS Number:: 3625-32000-061-00D
Scientists: Olsen, S., Bricker, B., Stoffregen, W., Halling, S., Whipple D.
Location: Ames, IA, National Animal Disease Center, Bacterial Diseases of Livestock
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PROJECT OBJECTIVES:
Brucellosis is a disease of cattle that causes abortion and associated economic losses and has been nearly eradicated from cattle in the U.S. after 60 years of regulatory efforts. However, a high percentage of feral swine, and elk and bison in Yellowstone National Park and surrounding areas have brucellosis and have the potential to transmit the disease to cattle. During the winter months, elk may share feed grounds with cattle, and bison may migrate from Yellowstone National Park to areas where they may have direct contact with cattle. We have identified *Brucella abortus* strain RB51 as an efficacious vaccine for bison and cattle that does not interfere with identification of *Brucella*-infected cattle. However, recent data suggest that the strain RB51 vaccine does not protect elk against brucellosis. Through studies at the USDA-ARS-National Animal Disease Center (NADC), Ames, IA, and collaborative work with scientists from other agencies, we are working to develop new vaccines that are safe and efficacious and vaccine delivery systems that would protect wildlife and domestic livestock against brucellosis. This will facilitate the completion of the Brucellosis Eradication program and assist in reducing or eradicating the disease from wildlife. ARS is also conducting research to determine the duration of immunity when cattle are vaccinated as calves with *B. abortus* strain RB51. This is important to determine how long cattle are protected against brucellosis. Several feral swine populations in the US are infected with *B. suis* and pose a potential threat to the livestock industry. ARS scientists are conducting research to develop efficacious vaccines to protect feral swine against *B. suis* infection and prevent transmission of the organisms to domestic livestock. In addition, the whole genome of a field strain of *B. abortus* is being sequenced to identify potential vaccine candidates and tools for molecular epidemiology.

OVERALL PROJECT ACCOMPLISHMENTS:
As a result of work first initiated in 1991, the *B. abortus* strain RB51 vaccine received full licensure from the USDA-Animal and Plant Health Inspection Service (APHIS) in 2003 as the first new brucellosis vaccine for cattle in over 50 years. Strain RB51 has replaced the strain 19 vaccine throughout the U.S. and is being used, or considered for use, in numerous other countries. The strain RB51 vaccine induces similar protection in cattle against brucellosis as the previously used strain 19 vaccine, and also has a significant advantage over strain 19 in that cattle vaccinated with strain RB51 do not have antibody responses that cause positive reactions on brucellosis surveillance tests. This has eliminated countless vaccine-induced, false-positive serologic responses that previously would have required investigation by regulatory personnel. Our studies have demonstrated that strain RB51 is safe in cattle or bison as a calfhood vaccine, is safe in adult bison bulls, and can be safely administered at a reduced dosage to pregnant cattle. Our data also demonstrate that calfhood vaccination of bison with strain RB51 protects against Brucella-induced abortion or fetal infection. With the completion of appropriate safety and efficacy studies in target and nontarget species, it is anticipated that strain RB51 will be utilized in bison in the Greater Yellowstone Area. Our studies have also suggested that antigens that stimulate protective cell-mediated immunity may be conserved across Brucella species. These data suggest that genes or

gene products which mediate protection against *B. abortus* will have efficacy in protection against other strains of brucellosis which are more prevalent in other countries.

A new method to determine and compare the DNA fingerprint of *Brucella* isolates was developed using information generated from the project to sequence the whole genome of *B. abortus.* This method has been used to help identify potential sources of infection and aid in epidemiological investigations of outbreaks of brucellosis in cattle.

IMPACT:
Results of ARS research were used to support granting full licensure for use of *B. abortus* strain RB51 as the first new brucellosis vaccine for cattle in 50 years. The vaccine is now used throughout the US and other parts of the world. The safety and efficacy of *B. abortus* strain RB51 is now being evaluated in bison for possible use in Yellowstone National Park.

The DNA fingerprinting method developed by ARS scientists is being used to aid state and federal regulatory officials in their investigations of outbreaks of brucellosis. This is the first method that can be used to reliably determine the fingerprint of *Brucella* strains beyond species and biovar level and represents an important advance in the field of science.

Research conducted by ARS scientists has led to the discovery of *B. abortus* infection in a population of feral swine that is also infected with *B. suis*. This is an important discovery and suggests that feral swine my pose a threat for transmission of both organisms to domestic livestock. Additional research is being conducted to determine the extent of the problem and to develop vaccines to protect feral swine against infection with *Brucella*.

TECHNOLOGY TRANSFER:
The *Brucella abortus* strain RB51 vaccine has been transferred to a company that is producing a commercial strain RB51 product which is approved by the Animal and Plant Health Inspection Service (APHIS). The RB51 vaccine is being universally utilized by producers throughout the U.S. and in a number of other countries. Through attendance and presentations at international meetings, national meetings, symposia, the Greater Yellowstone Interagency Brucellosis Committee, and local presentations, the results from our research have been presented to scientists, regulatory personnel and producers for field application. Knowledge on development of brucellosis vaccines and characterization of immunologic responses has also been transferred to Russian scientists through a cooperative program in the Department of Defense. Scientists are continuing to assist APHIS in completion of an environmental assessment of RB51. Knowledge gained by ARS scientists in regards to the use of the strain RB51 vaccine in cattle, bison, and elk has been transferred to state and federal regulatory personnel and is being incorporated into brucellosis management programs.

PUBLICATIONS:

Olsen SC, Holland SD. Safety of revaccination of pregnant bison with *Brucella abortus* strain RB51. J Wildl Dis. 2003; 39(4):824-9.

Bricker BJ, Ewalt DR, Halling SM. Brucella 'HOOF-Prints': strain typing by multi-locus analysis of variable number tandem repeats (VNTRs). BMC Microbiol. 2003; 3(1):15.

Bricker BJ, Ewalt DR, Olsen SC, Jensen AE. Evaluation of the *Brucella abortus* species-specific polymerase chain reaction assay, an improved version of the Brucella AMOS polymerase chain reaction assay for cattle. J Vet Diagn Invest. 2003; 15(4):374-8.

Ewalt DR, Bricker BJ. Identification and differentiation of *Brucella abortus* field and vaccinestrains by BaSS-PCR. Methods Mol Biol. 2003; 216:97-108.

Olsen SC, Jensen AE, Stoffregen WC, Palmer MV. Efficacy of calfhood vaccination with *Brucella abortus* strain RB51 in protecting bison against brucellosis. Res Vet Sci. 2003; 74(1):17-22.

Olsen SC, Kreeger TJ, Palmer MV. Immune responses of elk to vaccination with *Brucella abortus* strain RB51. J Wildl Dis. 2002; 38(4):746-51.

Olsen SC, Kreeger TJ, Schultz W. Immune responses of bison to ballistic or hand vaccination with *Brucella abortus* strain RB51. J Wildl Dis. 2002;38(4):738-45.

Kreeger TJ, DeLiberto TJ, Olsen SC, Edwards WH, Cook WE. Safety of *Brucella abortus* strain RB51 vaccine in non-target ungulates and coyotes. J Wildl Dis. 2002; 38(3):552-7.

Waters WR, Sacco RE, Fach SJ, Palmer MV, Olsen SC, Kreeger TJ. Analysis of mitogen-stimulated lymphocyte subset proliferation and nitric oxide production by peripheral blood mononuclear cells of captive elk (*Cervus elaphus*). J Wildl Dis. 2002; 38(2):344-51.

Bricker BJ. Diagnostic strategies used for the identification of Brucella.
Vet Microbiol. 2002; 90(1-4):433-4.

Bricker BJ. PCR as a diagnostic tool for brucellosis. Vet Microbiol. 2002; 90(1-4):435-46.

Halling SM. Paradigm shifts in vaccine development: lessons learned about antigenicity, pathogenicity and virulence of Brucellae. Vet Microbiol. 2002; 90(1-4):545-52.

Paulsen IT, Seshadri R, Nelson KE, Eisen JA, Heidelberg JF, Read TD, Dodson RJ, Umayam L, Brinkac LM, Beanan MJ, Daugherty SC, Deboy RT, Durkin AS, Kolonay JF, Madupu R, Nelson WC, Ayodeji B, Kraul M, Shetty J, Malek J, Van Aken SE, Riedmuller S, Tettelin H, Gill SR, White O, Salzberg SL, Hoover DL, Lindler LE, Halling SM, Boyle SM, Fraser CM. The *Brucella suis* genome reveals fundamental similarities between animal and plant pathogens and symbionts. Proc Natl Acad Sci U S A. 2002;99(20):13148-53.

Halling SM. Simple device to deliver beads to 96-well plates for rapid resuspension of bacterial pellets. Biotechniques. 2002; 33(3):480, 482.

Halling SM, Zuerner RL. Evidence for lateral transfer to Brucellae: characterization of a locus with a Tn-like element (Tn2020). Biochim Biophys Acta. 2002;1574(1):109-16.

Kreeger TJ, Cook WE, Edwards WH, Elzer PH, Olsen SC. *Brucella abortus* strain RB51 vaccination in elk. II. Failure of high dosage to prevent abortion. J Wildl Dis. 2002; 38(1):27-31.

Lee IK, Olsen SC, Bolin CA. Effects of exogenous recombinant interleukin-12 on immune responses and protection against *Brucella abortus* in a murine model. Can J Vet Res. 2001; 65(4):223-8.

Rhyan JC, Gidlewski T, Ewalt DR, Hennager SG, Lambourne DM, Olsen SC. Seroconversion and abortion in cattle experimentally infected with Brucella sp. isolated from a Pacific harbor seal (*Phoca vitulina richardsi*). J Vet Diagn Invest. 2001; 13(5):379-82.

Januszewski MC, Olsen SC, McLean RG, Clark L, Rhyan JC. Experimental infection of nontarget species of rodents and birds with *Brucella abortus strain* RB51 vaccine. J Wildl Dis. 2001; 37(3):532-7.

Redkar R, Rose S, Bricker B, DelVecchio V. Real-time detection of *Brucella abortus*, *Brucella melitensis* and *Brucella suis*. Mol Cell Probes. 2001;15(1):43-52.

Halling SM, Koster NA. Use of detergent extracts of *Brucella abortus* RB51 to detect serologic responses in RB51-vaccinated cattle. J Vet Diagn Invest. 2001; 13(5):408-12.

Bacterial Diseases of Livestock

Project Title: Understanding Host-Pathogen Interactions for the Diagnosis and Control of Paratuberculosis (Johne's Disease)
CRIS Number: 3625-32000-062-00D
Scientists: Stabel, J., Bannantine, J., Robbe Austerman, S., Paustian, M., Peterson-Burch, B., Whipple, D.
Location: Ames, IA, National Animal Disease Center, Bacterial Diseases of Livestock
Contact: Stabel, J., Phone: (515) 663-7304, Fax: (515) 663-7458, jstabel@nadc.ars.usda.gov

PROJECT OBJECTIVES:
Paratuberculosis (Johne's disease) is a chronic, progressive enteric disease of ruminants caused by infection with *Mycobacterium 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.

Research in improved diagnostic tests for detection of this disease will provide tools for reducing contamination of the environment with *M. paratuberculosis* and the spread of infection in a herd. Research on the pathogenesis and immunology of *M. paratuberculosis* infections of cattle is conducted to allow design of more rational diagnostic and control procedures. Sequencing the genome of *M. paratuberculosis* will lead to identification of specific antigens for *M. paratuberculosis* and will result in better diagnostic tests to allow early detection of subclinically infected animals and reduce the incidence of disease in herds.

OVERALL PROJECT ACCOMPLISHMENTS:
Significant progress has been made in the development and/or optimization of new diagnostic tools for the detection of Johne's disease. Fecal culture methodology has been improved and diagnostic laboratories throughout the U.S. are using this technique. Optimization of the gamma-interferon test for detection of subclinically infected animals has been achieved through incorporation of our antigen preparations in the test, allowing for detection of infected animals less than 6 months of age. The ability to accurately identify animals in the early stages of disease is a critical control point. Development of the fecal polymerase chain reaction (PCR) test may yield more timely and sensitive detection of infected animals that are shedding the bacteria in their feces.

Little information is available on host immune responses to *M. paratuberculosis* infection in cattle and other species of ruminants. Host immune responses in the subclinical and clinical stages of disease have been evaluated using cattle, bison, and a mouse model. Our work has shown that secretion of proteins (cytokines, IFN-g, IL-10, TGF-b) by immune cells and activation of immune cells is affected by the different stages of infection. This information is helpful in developing diagnostic tools for Johne's disease as well as treatment strategies.

M. paratuberculosis has been implicated as a causative factor in Crohn's disease, an inflammatory bowel disease, in humans. A proposed method of infection is via contaminated dairy products from cows with Johne's disease. Experiments were conducted to evaluate current pasteurization standards set by the dairy industry on the destruction of *M. paratuberculosis* in raw milk using state-of-the-art technology. Our work has demonstrated that continuous flow pasteurization of milk effectively destroys *M. paratuberculosis*. This work is critical in ensuring a safe, clean product for U.S. consumers.

Improvement of diagnostic tools and development of vaccines is dependent upon the identification of specific antigens for *M. paratuberculosis*. A genomic library for *M. paratuberculosis* was screened and several clones specific to *M. paratuberculosis* were recovered. One clone, HspX, has been characterized in our laboratory and has been patented. Thirty additional genes have been cloned and expressed and are being evaluated for use in diagnostic tests. Further characterization of additional clones and expression of proteins from them will aid in the development of more sensitive diagnostic tests.

IMPACT:
Results of ARS research showed that *M. paratuberculosis* is effectively killed at high concentrations in milk during pasteurization. In the study, several heat treatment regimes were evaluated using three different strains of *M. paratuberculosis*. Evaluation of current pasteurization methods used by the U.S dairy industry significantly impacted the recommendation that pasteurized milk is safe for human consumption.

Identification of new unique genes for *M. paratuberculosis* will have significant impact on development of diagnostic tools for detection of Johne's disease in ruminants. Currently, cross-reactivity with other mycobacterial species in serologic tests can result in false positive test results for Johne's disease, which causes economic loss for livestock producers. In addition, sensitivity of detection is a significant problem in most diagnostic tests for Johne's disease. Developing and optimizing improved diagnostic tests for identification of infected animals is important for controlling the spread of this disease. Furthermore, these antigens can be evaluated as vaccine candidates in the calves to either prevent infection or decrease clinical signs.

Continued work on host immune responses in different stages of disease (subclinical and clinical) will provide information on how the disease progresses and what causes the progression from the latent state to clinical stages of disease. This information can be used to develop new management strategies to prevent escalation of disease within a herd or treatment options that may be cost-effective for the producer.

TECHNOLOGY TRANSFER:
Polyclonal antibodies to preparations of *M. paratuberculosis* developed in our laboratory have been transferred to several companies (Cooperative Research and Development Agreements or MTAs) in collaborative efforts to develop new diagnostic tools for Johne's disease. Commercial availability of the new diagnostic tools is dependent upon the companies involved and successful use of these tools.

Invitations to speak about effects of pasteurization on destruction of *M. paratuberculosis* have resulted in the transfer of this information to the dairy industry and producers.

ARS scientists have transferred technology by giving numerous presentations on various aspects of Johne's disease research to scientific organizations and livestock industry groups. Groups have been particularly interested in research on pasteurization of milk, development of improved diagnostic tests, and application of the project to sequence the genome of *M. paratuberculosis*.

PUBLICATIONS:

Khalifeh MS, Stabel JR. Effects of Gamma Interferon, Interleukin-10, and Transforming Growth Factor beta on the Survival of *Mycobacterium avium* subsp. *paratuberculosis* in Monocyte-Derived Macrophages from Naturally Infected Cattle. Infect Immun. 2004; 72(4):1974-82.

Amonsin A, Li LL, Zhang Q, Bannantine JP, Motiwala AS, Sreevatsan S, Kapur V. Multilocus short sequence repeat sequencing approach for differentiating among *Mycobacterium avium* subsp. *paratuberculosis* strains. J Clin Microbiol. 2004; 42(4):1694-702.

Stabel JR, Bosworth TL, Kirkbride TA, Forde RL, Whitlock RH. A simple, rapid, and effective method for the extraction of *Mycobacterium paratuberculosis* DNA from fecal samples for polymerase chain reaction. J Vet Diagn Invest. 2004; 16(1):22-30.

Bannantine JP, Hansen JK, Paustian ML, Amonsin A, Li LL, Stabel JR, Kapur V. Expression and immunogenicity of proteins encoded by sequences specific to *Mycobacterium avium* subsp. *paratuberculosis*. J Clin Microbiol. 2004; 42(1):106-14.

Bannantine JP, Zhang Q, Li LL, Kapur V. Genomic homogeneity between *Mycobacterium avium* subsp. *avium* and *Mycobacterium avium* subsp. *paratuberculosis* belies their divergent growth rates. BMC Microbiol. 2003; 3(1):10.

Stabel JR, Goff JP, Kimura K. Effects of supplemental energy on metabolic and immune measurements in periparturient dairy cows with Johne's disease. J Dairy Sci. 2003; 86(11):3527-35.

Stabel JR, Palmer MV, Whitlock RH. Immune responses after oral inoculation of weanling bison or beef calves with a bison or cattle isolate of *Mycobacterium avium* subsp. *paratuberculosis*. J Wildl Dis. 2003; 39(3):545-55.

Waters WR, Miller JM, Palmer MV, Stabel JR, Jones DE, Koistinen KA, Steadham EM, Hamilton MJ, Davis WC, Bannantine JP. Early induction of humoral and cellular immune responses during experimental *Mycobacterium avium* subsp. *paratuberculosis* infection of calves. Infect Immun. 2003; 71(9):5130-8.

Bannantine JP, Huntley JF, Miltner E, Stabel JR, Bermudez LE. The *Mycobacterium avium* subsp. *paratuberculosis* 35 kDa protein plays a role in invasion of bovine epithelial cells. Microbiology. 2003; 149(Pt 8):2061-9.

Motiwala AS, Strother M, Amonsin A, Byrum B, Naser SA, Stabel JR, Shulaw WP,
Bannantine JP, Kapur V, Sreevatsan S. Molecular epidemiology of *Mycobacterium avium* subsp. *paratuberculosis*: evidence for limited strain diversity, strain sharing, and identification of unique targets for diagnosis. J Clin Microbiol. 2003; 41(5):2015-26.

Stabel JR, Ackermann MR. Temporal *Mycobacterium paratuberculosis* infection in T-cell receptor (TCR)-alpha and TCR-delta-deficient mice. Vet Immunol Immunopathol. 2002; 89(3-4):127-32.

Thornton CG, MacLellan KM, Stabel JR, Carothers C, Whitlock RH, Passen S. Application of the C (18)-carboxypropylbetaine specimen processing method to recovery of *Mycobacterium avium* subsp. *paratuberculosis* from ruminant tissue specimens. J Clin Microbiol. 2002; 40(5):1783-90.

Stabel JR, Wells SJ, Wagner BA. Relationships between fecal culture, ELISA, and bulk tank milk test results for Johne's disease in US dairy herds. J Dairy Sci. 2002; 85(3):525-31.

Bannantine JP, Stabel JR. Killing of *Mycobacterium avium* subspecies *paratuberculosis* within macrophages. BMC Microbiol. 2002; 2(1):2.

Lee H, Stabel JR, Kehrli ME Jr. Cytokine gene expression in ileal tissues of cattle infected with *Mycobacterium paratuberculosis*. Vet Immunol Immunopathol. 2001; 82(1-2):73-85.

Bannantine JP, Baechler E, Zhang Q, Li L, Kapur V. Genome scale comparison of *Mycobacterium avium* subsp. *paratuberculosis* with *Mycobacterium avium* subsp. *avium* reveals potential diagnostic sequences. J Clin Microbiol. 2002; 40(4):1303-10.

Bannantine JP, Stabel JR. Identification of two *Mycobacterium avium* subspecies *paratuberculosis* gene products differentially recognized by sera from rabbits immunized with live mycobacteria but not heat-killed mycobacteria. J Med Microbiol. 2001; 50(9):795-804.

Stabel JR, Whitlock RH. An evaluation of a modified interferon-gamma assay for the detection of paratuberculosis in dairy herds. Vet Immunol Immunopathol. 2001; 79(1-2):69-81.

Dargatz DA, Byrum BA, Barber LK, Sweeney RW, Whitlock RH, Shulaw WP, Jacobson RH, Stabel JR. Evaluation of a commercial ELISA for diagnosis of paratuberculosis in cattle. J Am Vet Med Assoc. 2001; 218(7):1163-6.

Pillai SR, Jayarao BM, Gummo JD, Hue EC, Tiwari D, Stabel JR, Whitlock RH. Identification and sub-typing of *Mycobacterium avium* subsp. *paratuberculosis* and *Mycobacterium avium* subsp. *avium* by randomly amplified polymorphic DNA.
Vet Microbiol. 2001; 79(3):275-84.

Stabel JR. On-farm batch pasteurization destroys *Mycobacterium paratuberculosis* in wastemilk. J Dairy Sci. 2001 Feb; 84(2):524-7.

Motiwala, A.S., Strother, M., Amonsin, A., Byrum, B., Naser, S.A., Stabel, J.R., Shulaw, W.P., Bannantine, J.P., Kapur, V., Sreevatsan, S. Molecular epidemiology of Mycobacterium avium subsp. Paratuberculosis: evidence for limited strain diversity, strain sharing, and identification of unique targets for diagnosis. Journal of Clinical Microbiology. 2003. v. 41. p. 2015-26.

Bannantine, J.P., Zhang, Q., Li, L.L., Kapur, V. Genomic homogeneity between Mycobacterium avium subsp, avium and Mycobacterium avium subsp. Paratuberculosis belies their divergent growth. BMC Microbiology. 2003. v. 3.

Bannantine, J.P., Huntley, J.F., Miltner, E., Stabel, J.R., Bermudez, L.E. The Mycobacterium avium subsp. Paratuberculosis 35 kDa protein plays a role in the invasion of bovine epithelial cells. Microbiology. 2003. v. 149. p. 2061-9.

Stabel JR.Transitions in immune responses to Mycobacterium paratuberculosis. Vet Microbiol. 2000 Dec 20;77(3-4):465-73.

Bannantine JP, Stabel JR.HspX is present within Mycobacterium paratuberculosis-infectedmacrophages and is recognized by sera from some infected cattle.Vet Microbiol. 2000 Oct 20;76(4):343-58.

Stabel JR.Johne's disease and milk: do consumers need to worry?J Dairy Sci. 2000 Jul;83(7):1659-63.

Stabel JR.Cytokine secretion by peripheral blood mononuclear cells from cowsinfected with Mycobacterium paratuberculosis.Am J Vet Res. 2000 Jul;61(7):754-60.

Bannantine JP, Griffiths RS, Viratyosin W, Brown WJ, RockeyDD.A secondary structure motif predictive of protein localization to the chlamydial inclusion membrane. Cell Microbiol. 2000 Feb;2(1):35-47.

Suchland RJ, Rockey DD, Bannantine JP, Stamm WE. Isolates of Chlamydia trachomatis that occupy nonfusogenic inclusions lack IncA, a protein localized to the inclusion membrane. Infect Immun. 2000 Jan;68(1):360-7.

Ellingson, J. L. E., Stabel, J. R., Bishai, W. R., Frothingham, R., and Miller, J. M. 2000. Evaluation of the Accuracy and Reproducibilityof a Practical PCR Panel Assay for Rapid Detection and Differentiationof Mycobacterium avium subsp. paratuberculosis. Mol. Cell. Probes 14:153-161.

Mwangi SM, Stabel J, Lee E, Kehrli ME, Taylor MJ.Expression and characterization of a recombinant soluble form of bovine tumor necrosis factor receptor type I.Vet Immunol Immunopathol. 2000 Dec 29;77(3-4):233-41.

Bacterial Diseases of Livestock

Project Title: Diagnosis and Control of Tuberculosis in Livestock and Wildlife
CRIS Number: 3625-32000-063-00D
Scientists: Palmer, M., Waters, W., Whipple, D.
Location: Ames, IA, National Animal Disease Center, Bacterial Diseases of Livestock
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PROJECT OBJECTIVES:

Bovine tuberculosis, which is caused by *Mycobacterium bovis*, is an infectious disease that affects many species of animals as well as human beings. Animals infected with *M. bovis* can shed organisms as they exhale or cough and in various secretions including saliva, milk, and urine. Elimination of animals infected with *M. bovis* is important to prevent the spread of disease among animals and to human beings. The U.S. initiated a program to eradicate tuberculosis from cattle in 1917 when the prevalence of disease was approximately 5%. In general, the eradication program has been successful and today, less than 0.002% of cattle are infected with *M. bovis*. However, a low prevalence of disease has persisted over the last 10-15 years and it has not been possible to eradicate bovine tuberculosis from the U.S. using available technology. Improved diagnostic tests and control measures are needed to detect and eliminate the few remaining cattle herds that have bovine tuberculosis. In addition, tuberculosis has been detected in captive deer and elk and in wild white-tailed deer, coyotes, raccoons, bear, bobcat, opossum, and fox. There is epidemiological and experimental evidence to suggest that tuberculosis has been transmitted from deer to cattle. The presence of tuberculosis in wildlife poses a serious threat to the national eradication program. Other countries with tuberculosis in wildlife species have been unable to eradicate the disease from the domestic livestock population. The Bovine Tuberculosis Research Project at the USDA-ARS-National Animal Disease Center (NADC), Ames, IA, is conducting research to develop a better understanding of the interactions between various host species and *M. bovis*, including immune response, antemortem diagnosis, prevention through vaccination, disease progression and interspecies transmission.

OVERALL PROJECT ACCOMPLISHMENTS:

A new test, the polymerase chain reaction (PCR) for detection and identification of *M. bovis* in tissue samples collected for microscopic examination was developed. When animals are slaughtered, meat inspectors collect tissue samples from animals that are suspected of having tuberculosis. Tissue samples are examined for microscopic evidence of tuberculosis and for the presence of organisms. Stains used to detect *M. bovis* in a tissue sample also stain other organisms making it impossible to identify the organism in the sample. The new test detects a specific piece of DNA that is present only in organisms that cause tuberculosis. The new test permits more accurate and rapid identification of animals with tuberculosis than was previously possible. The test is used extensively at the request of state and federal regulatory officials to confirm suspected cases of tuberculosis in animals. This technology has been transferred to USDA, APHIS.

A method differentiate strains of *M. bovis* was developed. Differences among various strains of *M. bovis* can be identified by using specific genetic markers. Using these markers, it is possible to determine if different animals are infected with a common strain or different strains of *M. bovis*. This information is used by epidemiologists to determine possible sources of infection in outbreaks of tuberculosis in animals. This technology has been transferred to USDA, APHIS.

An interferon gamma blood based assay for the diagnosis of tuberculosis in cattle is currently available. NADC scientists, in cooperation with USDA, APHIS and various state agencies evaluated the accuracy of this assay compared to traditional skin testing. Such research allowed the approval of the interferon gamma assay for use in cattle.

An experimental model of tuberculosis in white-tailed deer was developed using intratonsilar inoculation. The resulting disease is very similar to that observed in naturally infected deer. This model has been used extensively by NADC scientists to study the transmission of tuberculosis among deer and between deer and cattle and has demonstrated that deer can transmit *M. bovis* to other deer or cattle through indirect contact such as the sharing of feed. This model has also been used to develop and evaluate improved tests for diagnosis of tuberculosis in deer and to determine the effectiveness of vaccines to prevent infection.

Current surveys to determine the prevalence of tuberculosis in wild white-tailed deer are based on examination of tissues in the head to detect lesions. In a study that involved detailed examination of the entire deer carcass, we determined that about 35-50% of the infected deer did not have lesions in the head. This underestimation of disease prevalence should be considered when estimating prevalence through surveillance efforts that focus on examination of the head only.

A safe, reliable, and reproducible method of aerosol delivery of *Mycobacterium bovis* to cattle, or other large ruminants was developed and validated by scientists at NADC. Aerosol exposure is believed to be a primary means of *M. bovis* transmission between cattle. NADC scientists conducted the first and only studies on aerosol exposure of *M. bovis* in cattle. These studies have shown that this method of exposure results in lesions similar to those seen in naturally infected cattle. Such a delivery method is being used at NADC to investigate disease pathogenesis, transmission, immune responses, and vaccine efficacy and could be used to deliver other pathogens of cattle where aerosol delivery is critical. Aerosol exposure of deer to *M. bovis* did not result in disease similar to that seen in naturally infected deer, further supporting the hypothesis that transmission of *M. bovis* between deer involves routes such as sharing of feed.

The outbreak of tuberculosis in white-tailed deer in Michigan, identified in 1995, represents the first wildlife reservoir of *M. bovis* in North America and a serious threat to the federal bovine tuberculosis eradication effort. ARS scientists at NADC demonstrated that *M. bovis* can be transmitted between deer in close contact, through the sharing of feed and to deer fawns through oral infection with milk containing *M. bovis*. These results document that contaminated milk, in addition to saliva, nasal secretions, urine and feces may be involved in transmission of *M. bovis* between deer. Such information is vital for state and federal officials to make educated decisions concerning disease control strategies.

Currently, tuberculin skin testing is the most common method of antemortem testing of deer for tuberculosis; however, tuberculin skin testing of deer lacks specificity and requires the capture and handling of animals at least twice for testing. ARS scientists at NADC have investigated various blood-based assays for the diagnosis of tuberculosis in deer species common in North America. ARS scientists have shown that a commercially available assay for interferon gamma may be useful in white-tailed deer, reindeer, and possibly elk. After further work, this assay could potentially supplement or replace skin testing in captive Cervidae, decreasing animal handling and testing time. Such tests would decrease overall cost to producers, animal stress and time to

diagnosis. Serological assays have also been investigated and may be useful for screening animals in field situations.

IMPACT:
The technology for PCR testing of formalin fixed tissues has been transferred to major stakeholders, USDA, APHIS, NVSL. This testing has allowed more rapid confirmation of tuberculosis, allowing epidemiologic investigation to start much earlier than was previously possible. Quarantine times for producers are reduced and continued spread of disease through animal movements is minimized.

Methods for differentiation of *M. bovis* strains has been transferred to USDA, APHIS, NVSL and are used to aid in identification of the source of herd infections and trace movements of infected animals.

The experimental model of infection in white-tailed deer has been used extensively by ARS scientists to study disease pathogenesis, evaluation of diagnostic tests, vaccine efficacy and interspecies transmission. This model is currently being used by investigators in other countries (Canada) to examine tuberculosis in red deer and elk. Utilizing this model, NADC scientists have demonstrated the ability of deer to transmit *M. bovis* to other deer and cattle through indirect contact such as the sharing of feed. This information has been extensively used by Michigan wildlife and agricultural officials to lobby for the banning of supplemental feeding of wildlife to control the tuberculosis outbreak in northern Michigan. Subsequent epidemiological investigations have supported these findings and shown an association between supplemental feeding and disease prevalence.

Aerosol methods of infection with *M. bovis* have been used by ARS scientists to evaluate vaccine efficacy in cattle. These are the first studies to examine aerosol infection in cattle, the method considered to be the most likely means of transmission between cattle. Aerosol infection studies in deer conducted by ARS have demonstrated that white-tailed deer infected by aerosol inoculation do not develop disease similar to that seen in naturally infected deer. This information further supports the hypothesis that supplemental feeding and shared feed are primary means of disease transmission and supports state and federal efforts to discontinue the practice of supplemental feeding of wildlife.

Research on improved diagnosis of tuberculosis in Cervidae has resulted in a change in the interpretation of tuberculin skin testing results in reindeer by state and federal veterinarians. Based on ARS research a new scattergram was adopted by USDA, APHIS for interpretation of skin tests results in reindeer that results in decreased numbers of false positive results, decreased needles destruction of animals, and decreased cost to USDA. Research on blood based diagnostic assays has resulted in the recommendation by the USAHA in 2003 that the gamma interferon assay for deer be evaluated side by side with skin testing to determine if this blood based assay may be a suitable replacement for skin testing. Serological assays developed or evaluated by NADC scientists have been used to assist Michigan wildlife officials in screening of large numbers of trapped wild deer as an approach to selectively remove tuberculous deer.

Research by NADC scientists comparing traditional tuberculin skin testing and the gamma interferon test for cattle resulted in the adoption of the gamma interferon test as an officially approved test by USDA for the diagnosis of tuberculosis in cattle. Furthermore, the gamma interferon test can be used in place of the comparative cervical skin test. This test is now being widely used in states such as Michigan and Texas.

Research on an assay to detect nitric oxide as a means of antemortem diagnosis of tuberculosis was patented by ARS. Patent # 6,673,566. PC 0152.01. Another patent on the use of a recombinant protein for tuberculosis diagnosis is in the process of being filed.

Diseases at the interface of wildlife and domestic animals have become increasingly important. The study of infectious diseases in wildlife is costly and challenging and very few institutions are involved in such research involving agents requiring bio-containment. NADC has been visited by scientists from Plum Island, Winnipeg and other laboratories to observe how NADC scientists conduct such research. The impact of the overall wildlife research at NADC has been that NADC scientists are consulted regularly on research in wildlife in bio-containment involving BL-3 agents and foreign animal diseases.

TECHNOLOGY TRANSFER:
PCR of formalin fixed tissues transferred to USDA, APHIS, NVSL.

Technology to differentiate strains of *M. bovis* transferred to USDA, APHIS, NVSL.

The gamma interferon assay as well as serological assays have been used on numerous occasions to assist state and federal animal health officials to more rapidly and accurately diagnose *M. bovis* infection in deer and cattle.

NADC scientists have presented research findings on improved diagnostic tests for Cervidae to producer groups such as the North American Deer Farmers Association and the Reindeer Owner's and Breeders Association at their annual meetings.

NADC scientists have presented research findings on improved diagnosis of tuberculosis through use of the interferon gamma assay in cattle and deer to the United States Animal Health Association on various occasions.

NADC scientists have presented research findings on the transmission of *M. bovis* between deer and from deer to cattle at various meetings of Michigan wildlife and agricultural officials, the United States Animal Health Association and USDA, APHIS. These officials have used such findings to implement a ban on supplemental feeding of wildlife to control the tuberculosis outbreak in Michigan.

PUBLICATIONS: (2001-present)

Palmer MV, Waters WR, Whipple DL, Slaughter RE, Jones SL. Evaluation of an in vitro blood-based assay to detect production of interferon-gamma by *Mycobacterium bovis*-infected white-tailed deer (*Odocoileus virginianus*). J Vet Diagn Invest. 2004; 16(1):17-21.

Palmer MV, Waters WR, Whipple DL. Aerosol exposure of white-tailed deer (*Odocoileus virginianus*) to *Mycobacterium bovis*. J Wildl Dis. 2003; 39(4):817-23.

Waters WR, Palmer MV, Nonnecke BJ, Whipple DL, Horst RL. *Mycobacterium bovis* infection of vitamin D-deficient NOS2-/- mice.Microb Pathog. 2004; 36(1):11-7.

Waters WR, Nonnecke BJ, Foote MR, Maue AC, Rahner TE, Palmer MV, Whipple DL, Horst RL, Estes DM. *Mycobacterium bovis* bacille Calmette-Guerin vaccination of cattle: activation of bovine CD4+ and gamma delta TCR+ cells and modulation by 1,25-dihydroxyvitamin D3. Tuberculosis (Edinb). 2003;83(5):287-97.

Waters WR, Palmer MV, Whipple DL, Carlson MP, Nonnecke BJ. Diagnostic implications of antigen-induced gamma interferon, nitric oxide, and tumor necrosis factor alpha production by peripheral blood mononuclear cells from *Mycobacterium bovis*-infected cattle. Clin Diagn Lab Immunol. 2003; 10(5):960-6.

Waters WR, Miller JM, Palmer MV, Stabel JR, Jones DE, Koistinen KA, Steadham EM, Hamilton MJ, Davis WC, Bannantine JP. Early induction of humoral and cellular immune responses during experimental *Mycobacterium avium* subsp. *paratuberculosis* infection of calves. Infect Immun. 2003; 71(9):5130-8.

Nonnecke BJ, Waters WR, Foote MR, Horst RL, Fowler MA, Miller BL. In vitro effects of 1,25-dihydroxyvitamin D3 on interferon-gamma and tumor necrosis factor-alpha secretion by blood leukocytes from young and adult cattle vaccinated with *Mycobacterium bovis* BCG. Int J Vitam Nutr Res. 2003; 73(4):235-44.

Palmer MV, Waters WR, Whipple DL. Aerosol delivery of virulent *Mycobacterium bovis* to cattle. Tuberculosis (Edinb). 2002;82(6):275-82.

Waters WR, Palmer MV, Olsen SC, Sacco RE, Whipple DL. Immune responses of elk to *Mycobacterium bovis* bacillus Calmette Guerin vaccination. Vaccine. 2003; 28;21(13-14):1518-26.

Waters WR, Rahner TE, Palmer MV, Cheng D, Nonnecke BJ, Whipple DL.
Expression of L-Selectin (CD62L), CD44, and CD25 on activated bovine T cells.
Infect Immun. 2003; 71(1):317-26.

Palmer MV, Waters WR, Whipple DL. Milk containing *Mycobacterium bovis* as a source of infection for white-tailed deer fawns (*Odocoileus virginianus*). Tuberculosis (Edinb). 2002; 82(4-5):161-5.

Waters WR, Palmer MV, Whipple DL. *Mycobacterium bovis*-infected white-tailed deer (*Odocoileus virginianus*): detection of immunoglobulin specific to crude mycobacterial antigens by ELISA. J Vet Diagn Invest. 2002; 14(6):470-5.

Kaneene JB, VanderKlok M, Bruning-Fann CS, Palmer MV, Whipple DL, Schmitt SM, Miller R. Prevalence of *Mycobacterium bovis* infection in cervids on privately owned ranches. J Am Vet Med Assoc. 2002; 220(5):656-9.

Palmer MV, Gosch G, Lyon R, Waters WR, Whipple DL. Apoptosis in lymph node granulomas from white-tailed deer (*Odocoileus virginianus*) experimentally infected with *Mycobacterium bovis*. J Comp Pathol. 2002; 127(1):7-13.

Palmer MV, Whipple DL, Butler KL, Fitzgerald SD, Bruning-Fann CS, Schmitt SM. Tonsillar lesions in white-tailed deer (*Odocoileus virginianus*) naturally infected with *Mycobacterium bovis*. Vet Rec. 2002; 151(5):149-50.

Waters WR, Sacco RE, Fach SJ, Palmer MV, Olsen SC, Kreeger TJ. Analysis of mitogen-stimulated lymphocyte subset proliferation and nitric oxide production by peripheral blood mononuclear cells of captive elk (*Cervus elaphus*). J Wildl Dis. 2002; 38(2):344-51.

Waters WR, Palmer MV, Sacco RE, Whipple DL. Nitric oxide production as an indication of *Mycobacterium bovis* infection in white-tailed deer (*Odocoileus virginianus*). J Wildl Dis. 2002; 38(2):338-43.

Palmer MV, Waters WR, Whipple DL. Susceptibility of raccoons (*Procyon lotor*) to infection with *Mycobacterium bovis*. J Wildl Dis. 2002; 38(2):266-74.

Palmer MV, Waters WR, Whipple DL. Lesion development in white-tailed deer (*Odocoileus virginianus*) experimentally infected with *Mycobacterium bovis*. Vet Pathol. 2002; 39(3):334-40.

Palmer MV, Whipple DL, Waters WR. Tuberculin skin testing in white-tailed deer (*Odocoileus virginianus*). J Vet Diagn Invest. 2001; 13(6):530-3.

Waters WR, Nonnecke BJ, Rahner TE, Palmer MV, Whipple DL, Horst RL. Modulation of *Mycobacterium bovis*-specific responses of bovine peripheral blood mononuclear cells by 1,25-dihydroxyvitamin D(3). Clin Diagn Lab Immunol. 2001; 8(6):1204-12.

Palmer MV, Whipple DL, Waters WR. Experimental deer-to-deer transmission of *Mycobacterium bovis*. Am J Vet Res. 2001; 62(5):692-6.

Whipple DL, Palmer MV, Slaughter RE, Jones SL. Comparison of purified protein derivatives and effect of skin testing on results of a commercial gamma interferon assay for diagnosis of tuberculosis in cattle. J Vet Diagn Invest. 2001; 13(2):117-22.

Palmer, MV DL Whipple, JB Payeur, DP Alt, KJ Esch, CS Bruning-Fann, JB Kaneene. Naturally occurring tuberculosis in white-tailed deer. J Am Vet Med Assoc 2000;216:1921-1924.

Palmer,MV DL Whipple, SC Olsen. Cell mediated and humoral immune responses in white-tailed deer experimentally infected with Mycobacterium bovis. Res Vet Sci 2000;68:95-98.

Waters, W.R., M.V. Palmer, B.A. Pesch, S.C. Olsen, M.J. Wannemuehler, and D.L. Whipple. 2000. MHC class II-restricted, CD4+ T cell proliferative responses of peripheral blood mononuclear cells from Mycobacterium bovis-infected white-tailed deer. Veterinary Immunology and Immunopathology, 76:215-229.

Waters, W.R., M.V. Palmer, B.A. Pesch, S.C. Olsen, M.J. Wannemuehler, and D.L. Whipple. 2000. Lymphocyte subset proliferative responses of Mycobacterium bovis-infected cattle to purified protein derivative. Veterinary Immunology and Immunopathology, 77:257-273.

Waters, W.R., R. Hontecillas, R.E. Sacco, F.A. Zuckermann, K.R. Harkins, J. Bassaganya-Riera, and M.J. Wannemuehler. 2000. Antigen-specific proliferation of porcine CD8aa cells to anextracellular bacterial pathogen. Immunology, 101: 333-341.

Virus and Prion Diseases of Livestock

Project Title: Emerging Viral Diseases of Swine
CRIS Number: 3625-32000-071-00D
Scientists: Lager, K., Cheung, A., Kehrli, M., Vacant
Location: Ames, IA, National Animal Disease Center, Virus and Prion Diseases of Livestock
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PROJECT OBJECTIVES:

Recently, swine influenza virus (SIV) has changed or mutated into different strains or subtypes that have caused significant losses for the swine industry. There are at least several SIV subtypes circulating in U.S. swine and currently available vaccines may not protect against all of the subtypes. In addition to the SIV concerns there has emerged a new swine virus, porcine circovirus type 2 (PCV2), that is associated with a newly recognized disease syndrome that causes significant losses for the industry, especially in young pigs. The overall goal of this project is to better understand how SIV and PCV2 cause disease in swine and then apply this knowledge to develop strategies that can protect swine from these viruses and control the disease that they cause.

OVERALL PROJECT ACCOMPLISHMENTS:

An inactivated swine influenza virus (SIV) vaccine was developed and this technology was transferred to a biologics company that is now marketing the vaccine to reduce the economic impact of swine flu. Rapid diagnostic tests have been developed for the detection of SIV, PCV2, and porcine reproductive and respiratory syndrome virus (PRRSV) and validation of these assays is underway. Recent studies have demonstrated Adenovirus-vectored SIV vaccines in young pigs may be used to circumvent the inhibitory effect of passively acquired SIV maternal antibody, and a potential attenuated SIV virus vaccine has been developed. Porcine circovirus studies have characterized the molecular biology of the virus resulting in a better understanding of how this virus replicates and future experiments will apply this knowledge towards understanding how the virus infects swine and causes disease.

IMPACT:

Use of the inactivated vaccine by pork producers has aided in the prevention and control of swine influenza. The rapid diagnostic tests can increase the efficincy of diagnostics and may decrease the cost of diagnostic work. Pathogenesis studies have indicated that even within the same SIV subtype (H3 subtype) there are antigenically distinct groups of virus and a single H3 vaccine strain may not cross-protect against all H3 SIV subtypes. This finding may explain potential vaccine failures and it indicates a potential need for continually evaluating SIV vaccine strains. Understanding the replication of porcine circovirus will provide a means to evaluate how this virus can cause disease and insight into possible ways the virus could be changed to produce a vaccine for prevention of disease.

TECHNOLOGY TRANSFER:
Transfer of SIV vaccine to a biologics company that licensed the vaccine and is currently selling it. Transfer PRRSV and SIV real-time reverse transcription-polymerase chain reaction (RT-PCR) assays to North Carolina State, Iowa State, and Oregon State Veterinary Diagnostic Laboratories for use in routine diagnostics.

PUBLICATIONS:

Richt, J.A., Lager, K.M., Clouser, D., Spackman, E., Suarez, D.L., Yoon, K-J. 2004. Real-time RT-PCR assays for the detection and differentiation of North American swine influenza viruses. *J. Vet. Diagn. Invest.,* in press

Cheung, A.K. Comparative analysis of the transcriptional patterns of pathogenic and nonpathogenic porcine circoviruses. Virology. 2003. v. 310. p. 41-49.

Richt, J.A., Lager, K.M., Janke, B.H., Woods, R.D., Webster, R.G. Webby, R.J. Pathogenic and antigenic properties of phylogenetically distinct reassortant H3N2 swine influenza viruses cocirculating in the United States. Journal of Clinical Microbiology. 2003. v. 41. p. 3198-3205.

Tang, M, Harp, J.A., Wesley, R.D. Recombinant adenovirus encoding the HA gene from swine H3N2 influenza virus partially protects mice from challenge with heterologous virus: A/HK/1/68 (H3N2). Archives of Virology. 2002. v. 147. p. 2125-2141.

Wesley, R. Neutralizing antibody decay and lack of contact transmission after inoculation of 3- and 4-day-old piglets with porcine respiratory coronavirus. Journal Veterinary Diagnostic Investigation. 2002. v. 14. p. 525-527.

Cheung, A.K. Transcriptional analysis of porcine circovirus type 2. Virology. 2003. v. 305. p. 168-180.

Cheung, A.K., Bolin, S.R. Kinetics of porcine circovirus type 2 replication. Archives of Virology. 2002. v. 147. p. 43-58.

Nawagitgul, P., Morozov, I., Bolin, S.R., Harms, P.A., Sorden, S.D., Paul P.S. Open reading frame 2 of porcine circovirus type 2 encodes a major capsid protein. Journal of General Virology. 2000. v. 81(Pt 9). p. 2281-2287.

---------- Additional publications by scientist in this project. ----------

Woods, R.D. Efficacy of a transmissible gastroenteritis coronavirus with an altered ORF-3 gene. Canadian Journal Veterinary Research. 2001. v. 65(1). p. 28-32.

Wesley, R., Lager, K. Increased litter survival rates, reduced clinical illness and better lactogenic immunity against TGEV in gilts that were primed as neonates with porcine respiratory coronavirus (PRCV). Vet. Micro. 95:175-186. (2003).
Stabel, T.J., Bolin, S.R., Pesch, B.A., Rahner, T.E. A simple and rapid flow cytometric method for detection of porcine cell surface markers. Journal of Immunologic Methods. 2000. v. 245(1-2). p. 147-152.

Virus and Prion Diseases of Livestock

Project Title: Virus-Induced Respiratory and Reproductive Diseases of Swine
CRIS Number:: 3625-32000-057-00D
Scientists: Wesley, R., Lager, K., Kehrli, M.
Location: Ames, IA, National Animal Disease Center, Virus and Prion Diseases of Livestock
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PROJECT OBJECTIVES:

Porcine reproductive and respiratory syndrome (PRRS) is a virus-induced disease of swine that is characterized clinically by reproductive failure of gilts and sows and a respiratory tract illness that is especially severe in young pigs. It was discovered first in the U.S. in 1987 and soon spread worldwide. The sudden emergence of PRRS virus in the swine population has led to speculation that it may have arisen by mutation of a similar virus of another species. However, as of today the origin of PRRS virus is still in question. Our current research is focused on developing methods for diagnosing, preventing, controlling, and eventually eradicating this costly disease.

OVERALL PROJECT ACCOMPLISHMENTS:

Accomplishments over the life of the project include: 1) developing better methods of diagnosing PRRS; 2) developing, evaluating and licensing a laboratory procedure for identifying strains of PRRS virus; 3) developing, evaluating and licensing a new vaccine for the prevention of atypical PRRS; 4) defining when and how PRRS virus causes disease; and 5) providing service and consultation to industry representatives, swine practitioners, other researchers, and swine producers in their efforts to prevent and control PRRS.

IMPACT:

The vaccine is being used by pork producers to prevent and control PRRS. The diagnostic assay has been used extensively by diagnosticians and field veterinarians for differentiating PRRSV isolates. The animal studies have provided insight into the pathogenesis of the virus and this knowledge is being used to develope and improve PRRS control and prevention strategies.

TECHNOLOGY TRANSFER:

A unique PRRSV isolate was developed into an attenuated vaccine and transferred to a biologics company for liscensing and marketing. Likewise a unique diagnostic assay was developed that can be used to differentiate PRRSV isolates by a simple code, this technology is used daily by veterinarians and pork producers. Formal collaborations between ARS and industry has provided information about the safety and efficacy of PRRSV vaccines and this information has been applied by veterinarians in the development of PRRS control and prevention strategies. Information concerning the use of replication defective human adenovirus-5 vaccines to overcome maternal antibody interference was provided to the National Pork Board and the International Pig Veterinary Society for use by their members. A Cooperative Research and Development Agreement with an international veterinary biologics company is being initiated to investigate these vectors or others to overcome this vaccine interference problem.

PUBLICATIONS:

Lemke, C.D., Haynes, J.S., Spaete, R., Adolphson, D., Vorwald, A., Lager, K., Butler, J.E. Lymphoid hyperplasia resulting in immune dysregulation is caused by porcine reproductive and respiratory syndrome virus infection in neonatal pigs. The Jouirnal of Immunology, 2004. v. 172. pp. 1916-1925.

Grebennikova, T.V., Clouser, D.F., Vorwald, A.C., Musienko, M.I., Mengeling, W.L., Lager, K.M., Wesley, R.D., Biketov, S.F., Zaberezhny, A.D., Aliper, T.I., Nepoklonov, E.A. Genomic characterization of virulent, attenuated, and revertant passages of a North American porcine reproductive and respiratory syndrome virus strain. Virology, 2004. v. 321. pp. 383-90.

Lager, K.M., Mengeling, W.L., Wesley, R.D. Strain predominance following exposure of vaccinated and naive pregnant gilts to multiple strains of porcine reproductive and respiratory syndrome virus. Canadian Journal of Veterinary Research. 2003. v. 67. p. 121-127.

Mengeling, W.L., Lager, K.M., Vorwald, A.C., Clouser, D.F. Comparative safety and efficacy of attenuated single-strain and multi-strain vaccines for porcine reproductive and respiratory syndrome. Veterinary Microbiology. 2003. v. 93. p. 25-38.

Mengeling, W.L., Lager, K.M., Vorwald, A.C., Koehler, K.J. Strain specificity of the immune response of pigs following vaccination with various strains of porcine reproductive and respiratory syndrome virus. Veterinary Microbiology. 2003. v. 93. p. 13-24.

Brockmeier, S.L., Lager, K.M. Experimental airborne transmission of porcine reproductive and respiratory syndrome virus and Bordetella bronchiseptica. Veterinary Microbiology. 2002. v. 89. p. 267-75.

Butler, J.E., Sun, J., Weber, P., Ford, S.P., Rehakova, Z., Sinkora, J., Francis, D., Lager, K. Switch recombination in fetal porcine thymus is uncoupled from somatic mutation. Veterinary Immunology and Immunopathology. 2002. v. 87. p. 307-319.

Wesley, R.D., and Lager, K.M. Increased litter survival rates, reduced clinical illness and better lactogenic immunity against TGEV in gilts that were primed as neonates with porcine respiratory coronavirus. Veterinary Microbiology. 2003. v. 95. pp. 175-186.

Lager K., Mengeling W., Wesley R. Evidence for local spread of porcine reproductive and respiratory syndrome virus. J. Swine Health Prod. 10:167-170 (2003).

Mengeling, W. L., Wesley, R. D., Lager, K. M., and Vorwald, A. C. Effect of concurrent infections on persistence and shedding of porcine reproductive and respiratory syndrome virus and transmissible gastroenteritis virus. J. Swine Health Prod. 2002. v. 10. pp. 67-73.

Mengeling, W. L., Boilin, S. R., Lager, K. M., Vorwald, A. C., and Wesley, R. D. Immunology and epidemiology of porcine reproductive and respiratory syndrome, pseudorabies and postweaning multisystemic wasting syndrome. Magazyny Weterynaryjny. 2002. supplement pigs, pp. 27-33.

Lager, K. M., Mengeling, W. L., and Wesley, R. D. Evidence for local spread of porcine reproductive and respiratory syndrome virus. J. Swine Health Prod. 2002. v. 10. pp. 167-170.

Benson, J. E., Yaeger, M. J., Christopher-Hennings, J., Lager, K., Yoon, K-J. A comparison of virus isolation, immunohistochemistry, fetal serology, and reverse-transcription polymerase chain reaction for the identification of porcine reproductive and respiratory syndrome virus transplacental infection in the fetus. J. Vet. Diagn. Invest. 14:8-14. (2002).

Mengeling, W.L., Lager, K.M., Burkhart, K, Vorwald, A.C., Roof, M.B., Gorcyca, D.E. Porcine reproductive and respiratory syndrome virus vaccine based on isolate JA-142. U.S. Patent Application S/N 09/298,110.

Brockmeier, S.L., Palmer, M.V., Bolin, S.R., Rimler, R.B. Effects of intranasal inoculation with Bordetella bronchiseptica, porcine reproductive and respiratory syndrome virus, or a combination of both organisms on subsequent infection with Pasteurella multocida in pigs. American Journal of Veterinary Research. 2001. v. 62(4). p. 521-525.

Brockmeier, S.L., Palmer, M.V., Bolin, S.R. Effects of intranasal inoculation of porcine reproductive and respiratory syndrome virus, Bordetella bronchiseptica, or a combination of both organisms in pigs. American Journal of Veterinary Research. 2000. v. 61(8). p. 892-899.

Mengeling, W.L., Vorwald, A.C., Cornick, N.A., Lager, K.M., Moon, H.W. In vitro detection of Shiga toxin using porcine alveolar macrophages. Journal of Veterinary Diagnostic Investigation. 2001. v. 13. p. 421-424.

Harms, P.A., Sorden, S.D., Halbur, P.G., Bolin, S.R., Lager, K.M., Morozov, I., Paul, P.S. Experimental reproduction of severe disease in CD/CD pigs concurrently infected with type 2 porcine circovirus and porcine reproductive and respiratory syndrome virus. Veterinary Pathology. 2001. v. 38. p. 528-539.

Mengeling, W. L., Lager, K. M., Vorwald, A. C., Wesley, R. D. and Prieto, C. Porcine reproductive and respiratory syndrome: vaccines and immunity. Animal Reprod. Sci. 2000. v. 60-61. pp.199-210.

Mengeling, W. L., Lager, K. M. A brief review of procedures and potential problems associated with the diagnosis of porcine reproductive and respiratory syndrome. 2000. Veterinary Research. v. 31(1). p. 61-69.

Mengeling, W. L., and Lager, K. M. A brief review of procedures and potential problems associated with the diagnosis of porcine reproductive and respiratory syndrome. Vet. Res. 31:61-69. (2000).

Lager, K.M., Mengeling, W. L. Diagnosing acute infections of porcine reproductive and respiratory disease syndrome in swine. Vet. Res. 31:70. (2000).

Mengeling, W. L., Lager, K. M., Vorwald, A. C. The effect of porcine parvovirus and porcine reproductive and respiratory syndrome virus on porcine reproductive performance. Jul 2000. Animal Reproduction Science. v. 60-61. p. 199-210.

Wesley, R. D., Clouser, D. F., Mengeling, W. L., Andreyev, V. G., Vorwald, A. C., Lager, K. M. Restriction enzyme screen for differentiating porcine reproductive and respiratory syndrome virus strains. Jan 2000. U.S. Patent 6,015,663.

---------- Additional publications by scientist in this project. ----------

Soucie, J. M., Erdman, D. D., Evatt, B. L., Anderson, L. J., Torok, T. J., El-Jamil, M. Barnhart, E. Tepper, M. Burrill, H. N., Pickett, A. M., Mengeling, W. L. Investigation of porcine parvovirus among persons with hemophilia receiving Hyate:C porcine factor VIII concentrate. Jun 2000. Transfusion. v. 40(6). p. 708-711.

Cheung, A.K., Chen, Z., Sun, Z., McCullough, D. Pseudorabies virus induces apoptosis in tissue culture cells. Archives of Virology. 2000. v. 145. p. 2193-2200.

Virus and Prion Diseases of Livestock

Project Title: Detection and Control of Bovine Viral Diarrhea Viruses
CRIS Number: 3625-32000-065-00D
Scientists: Ridpath, J., Neill, J., Vacant
Location: Ames, IA, National Animal Disease Center, Virus and Prion Diseases of Livestock
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PROJECT OBJECTIVES:
Bovine viral diarrhea viruses (BVDV) acting alone or in conjunction with other pathogens are a source of major economic loss to the U.S. farm economy. Pathogenesis varies from strain to strain and appears to be the result of a very complex interaction between virus and host. Variation in virulence is not correlated with replication rates or cytopathology in vitro. Pathogenesis appears to be due in part to the host's response to viral replication rather than viral replication itself. The goals of this project are to improve the industry's ability to limit and control infections. Our approach is to correlate variations among BVDV with differences in immune response and pathogenesis.

OVERALL PROJECT ACCOMPLISHMENTS:
This is a project based on a Project Plan certified by the OSQR February 2, 2002. Current research activity follows from the previous project. Major accomplishments for the proceeding project include the recognition of a new type of BVDV (genotype BVDV2). BVDV2 strains differ from previously described BVDV strains (genotype BVDV1) in the way they are recognized by the immune system, in the makeup of their genetic information and in the diseases they cause. It was shown that differences between BVDV2 and BVDV1 strains result in diagnostic and vaccine failure. Further, it was shown that viruses, which cause the most severe form of acute BVDV infection, belong to the BVDV2 genotype. The first complete characterization of the genetic makeup of BVDV2 strains was performed. In response to suggestions that BVDV2 strains represented a variation of the bovine border disease virus (BDV) the first complete characterization of a BDV genome was done. Comparison of the genetic makeup of BVDV1, BVDV2 and BDV viruses demonstrated that BVDV2 strains were more closely related to BVDV1 strains than BDV strains. Tests based on these comparisons were developed that differentiated BVDV2 strains from BVDV1 and BDV strains. These tests are now standard protocol at numerous state diagnostic laboratories and the USDA-Animal and Plant Health Inspection Services (APHIS), National Veterinary Services Laboratory (NVSL) Diagnostic Virology Laboratory (DVL), Ames, IA. Two new BVDV vaccines, that are the result of collaboration between NADC and commercial biologics companies, were recently released. These vaccines offer a wider spectrum of protection compared to previous vaccines.

IMPACT:
Research done in this project has resulted in a new generation of diagnostics and vaccine that do a better job of detecting and controlling BVDV infections.

TECHNOLOGY TRANSFER:
Both scientist were on the organizing committee for the meeting “Detecting and Controlling BVDV Infections held in Ames, IA April 4-5, 2002. The purpose of this meeting was to foster discussion and interaction

between researchers, diagnosticians, practitioners and biologics company employees regarding strategies aimed at reducing BVDV losses.

Both scientists acted as editors and contributed articles for a special volume of the journal Biologicals that was devoted to issues involved with detecting, limiting and preventing BVDV infections. The target audience for this volume was diagnosticians and field veterinarians.

In conjunction with Agrilabs produced a compact disk that included information on the significance of BVDV genotypes and biotypes to BVDV management programs. This CD was generated for distribution to producers and field veterinarians.

Attended meeting and contributed to proceeding of meeting (Paris, March 30, 2001) hosted by Office International des Epizooties (OIE) called to discuss international standards for purity of reagents used in biologics production. Asked to speak on BVDV contamination of fetal calf serum.

Conducted classes for veterinarians and producers on the basic biology of BVDV, impact of BVDV and development of BVDV vaccines for producers and veterinarians (classes qualified as 2 to 3 hours of continuing education credits for attending veterinarians): Breckenridge, Colorado – July 10, 2002; Reno, Nevada - February 28, 2003; Yerington, Nevada - March 1, 2003; Breckenridge, Colorado - July 9, 2003; Hot Springs, Arkansas – Nov. 21, 2003; Scottsdale, Arizona – January 27, 2004; Oscoela, Iowa – April 7, 2004.

Contacted authors and reviewed manuscripts for a 4 part series on BVDV published in Hoard's Dairyman in 2002- 2003. Authored one article entitled BVDV: The Basics and co-authored another entitled "To Get Ahead of BVD: find out what strains you have, keep careful records, and take measures to prevent spread of all diseases".

Served as presenters at two "miniconferences" on BVDV held in conjunction with the National Cattlemen's Beef Association meeting (Cattlemen's College, Phoenix, Arizona - 1/28/04) and the Western Veterinary Conference (BVDV Control, Las Vegas, Nevada – 2/15/04).

Researchers in this project have provided numerous groups with one or more of the following: protocols, virus, antibodies, virus characterization, SAGE techniques and tag IDs, PCR primer sequences, genomic sequences and/or sequence analysis. These groups include: Biocor Animal Health; Centers for Disease Control and Prevention; Center for Veterinary Biologics and Product Development; Colorado Serum Company; Colorado State University; Cornell College of Veterinary Medicine; Fort Dodge Animal Health; Intervet, Inc.; Iowa State University; National Veterinary Services Laboratory; OIC Virology Laboratory, NSW, Australia; Oklahoma State University; University of California at Davis; University of Nebraska; University of North Dakota; Washington State University; and other research projects at NADC.

In addition, researchers in this group have participated in 3 Cooperative Research and Development Agreement (CRADA) with industry; 2 USDA-CSREES-NRI-CGP grant; attended and presented data at 4 international meetings, 11 national meetings and 5 regional meetings; acted as outside reviewers for the Office International des Epizooties (OIE) Manual on BVDV; and acted as scientific reviewers for the following journals: Biologicals; Archives of Virology Comparative Immunology; Microbiology and Infectious Diseases; Journal

of Clinical Microbiology; Journal of Virology; Veterinary Microbiology; and Virus Research, Virology and served on the editorial board of Virus Research and Veterinary Research Communications.

PUBLICATIONS:

Chen, R., Neill, J.D., Venkataram Prasad, B.V. Crystallization and preliminary crystallographic analysis of San Miguel sea lion virus: An animal calicivirus. Journal of Structural Biology. 2003. v. 141. p. 143-148.

Endsley JJ, Ridpath JF, Neill JD, et al. Induction of T lymphocytes specific for bovine viral diarrhea virus in calves with maternal antibody. *Viral Immunol* 2004;17:13-23.

Endsley JJ, Roth JA, Ridpath J, et al. Maternal antibody blocks humoral but not T cell responses to BVDV. *Biologicals* 2003;31:123-125.

Evermann JF, Ridpath JF. Clinical and epidemiologic observations of bovine viral diarrhea virus in the northwestern United States. *Vet Microbiol* 2002;89:129-139.

Flores EF, Gil LH, Botton SA, et al. Clinical, pathological and antigenic aspects of bovine viral diarrhea virus (BVDV) type 2 isolates identified in Brazil. *Vet Microbiol* 2000;77:175-183.

Flores EF, Ridpath JF, Weiblen R, et al. Phylogenetic analysis of Brazilian bovine viral diarrhea virus type 2 (BVDV-2) isolates: evidence for a subgenotype within BVDV-2. *Virus Res* 2002;87:51-60.

Fulton RW, Briggs RE, Payton ME, et al. Maternally derived humoral immunity to bovine viral diarrhea virus (BVDV) 1a, BVDV1b, BVDV2, bovine herpesvirus-1, parainfluenza-3 virus bovine respiratory syncytial virus, Mannheimia haemolytica and Pasteurella multocida in beef calves, antibody decline by half-life studies and effect on response to vaccination. *Vaccine* 2004;22:643-649.

Fulton RW, Ridpath JF, Confer AW, et al. Bovine viral diarrhoea virus antigenic diversity: impact on disease and vaccination programmes. *Biologicals* 2003;31:89-95.

Fulton RW, Ridpath JF, Saliki JT, et al. Bovine viral diarrhea virus (BVDV) 1b: predominant BVDV subtype in calves with respiratory disease. *Can J Vet Res* 2002;66:181-190.
Fulton RW, Saliki JT, Confer AW, et al. Bovine viral diarrhea virus cytopathic and noncytopathic biotypes and type 1 and 2 genotypes in diagnostic laboratory accessions: clinical and necropsy samples from cattle. *J Vet Diagn Invest* 2000;12:33-38.

Fulton RW, Step DL, Ridpath JF, et al. Response of calves persistently infected with noncytopathic bovine viral diarrhea virus (BVDV) subtype 1b after vaccination with heterologous BVDV strains in modified live virus vaccines and Mannheimia haemolytica bacterin-toxoid. *Vaccine* 2003;21:2980-2985.

Green, K.Y. Ando, T., Balayan, M.S., Berke, T., Clarke, I.N., Estes, Matson, D.O., Makata, S., Neill, J.D., Studdert, M.J. and Thiel, H,J, Taxonomy of the caliciviruses. J Infect Dis 2000 181:S322-30.

Goyal SM, Bouljihad M, Haugerud S, et al. Isolation of bovine viral diarrhea virus from an alpaca. *J Vet Diagn Invest* 2002;14:523-525.

Liebler-Tenorio EM, Ridpath JE, Neill JD. Distribution of viral antigen and development of lesions after experimental infection with highly virulent bovine viral diarrhea virus type 2 in calves. *Am J Vet Res* 2002;63:1575-1584.

Liebler-Tenorio EM, Ridpath JF, Neill JD. Distribution of viral antigen and development of lesions after experimental infection of calves with a BVDV 2 strain of low virulence. *J Vet Diagn Invest* 2003;15:221-232.

Liebler-Tenorio EM, Ridpath JF, Neill JD. Lesions and tissue distribution of viral antigen in severe acute versus subclinical acute infection with BVDV2. *Biologicals* 2003;31:119-122.

Neill, J.D., Sosnovtsev, S.V. and Green, K.Y. Recovery and altered neutralization specificites of chimeric viruses containing capsid protein domain exchanges from antigenically distinct strains of feline calicivirus. J. Virol 2000:74:1079-1084.

Neill, J.D. The subgenomic RNA of feline calicivirus in packaged into viral particle during infection. Virus Res 2002:87:89-93.

Neill JD, Ridpath JF. Recombination with a cellular mRNA encoding a novel DnaJ protein results in biotype conversion in genotype 2 bovine viral diarrhea viruses. *Virus Res* 2001;79:59-69.

Neill JD, Ridpath JF. Gene expression changes in BVDV2-infected MDBK cells. *Biologicals* 2003;31:97-102.

Ridpath JE, Neill JD, Endsley J, et al. Effect of passive immunity on the development of a protective immune response against bovine viral diarrhea virus in calves. *Am J Vet Res* 2003;64:65-69.

Ridpath JF. BVDV genotypes and biotypes: practical implications for diagnosis and control(1). *Biologicals* 2003;31:127-131.

Ridpath JF, Hietala SK, Sorden S, et al. Evaluation of the reverse transcription-polymerase chain reaction/probe test of serum samples and immunohistochemistry of skin sections for detection of acute bovine viral diarrhea infections. *J Vet Diagn Invest* 2002;14:303-307.

Ridpath JF, Neill JD. Detection and characterization of genetic recombination in cytopathic type 2 bovine viral diarrhea viruses. *J Virol* 2000;74:8771-8774.

Ridpath JF, Neill JD, Frey M, et al. Phylogenetic, antigenic and clinical characterization of type 2 BVDV from North America. *Vet Microbiol* 2000;77:145-155.

Stoffregen B, Bolin SR, Ridpath JF, et al. Morphologic lesions in type 2 BVDV infections experimentally induced by strain BVDV2-1373 recovered from a field case. *Vet Microbiol* 2000;77:157-162.

Van Campen H, Ridpath J, Williams E, et al. Isolation of bovine viral diarrhea virus from a free-ranging mule deer in Wyoming. *J Wildl Dis* 2001;37:306-311.

Vilcek S, Ridpath JF, Van Campen H, et al. Identification of a novel pestivirus genotype isolated from pronghorn antelope. *American Society for Virology Annual Meeting*. American Society for Virology 20th Annual Meeting: American Society for Virology, 2001.

Virus and Prion Diseases of Livestock

Project Title: Bovine Spongiform Encephalopathy and Other Transmissible Spongiform Encephalopathies
CRIS Number: 3625-32000-066-00D
Scientists: Hamir, A., Kunkle, R., Richt, J., Kehrli, M., Vacant, Vacant
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PROJECT OBJECTIVES:
This project was expanded in January 2002 from the original TSE project that investigated scrapie. Today the project focuses on investigations of various Transmissible Spongiform Encephalopathies (TSE), which are fatal degenerative diseases of the central nervous system that can affect several animal species, including humans. The causal agent is believed to be a natural tissue protein, the prion protein, that has assumed an unnatural form. Because the altered protein is resistant to enzyme degradation, it accumulates in nervous tissue and the resulting dysfunction ultimately leads to death. The specific TSEs being investigated in this project are scrapie in sheep**,** chronic wasting disease (CWD) in deer and elk, and transmissible mink encephalopathy (TME) in mink. The major concern about these diseases is that another TSE, bovine spongiform encephalopathy (BSE), has been shown to cross the species barrier to cause a unique TSE in human beings. Although there has not been a similar demonstration that scrapie**,** CWD or TME could present any risk to human health, the BSE experience has raised many questions about the potential hazard these TSEs present for transmission to other animal species, especially domesticated livestock and wildlife. The major objectives are to assess transmissibility of the TSEs that affect livestock and wildlife species, to develop methods for differentiation of TSE strains, and to determine the pathobiology of these diseases in the natural host and after cross-species transmission. Results of animal inoculation studies are then compared to results obtained with a variety of laboratory procedures to determine if they can be used as predictive models for future risk assessments. These studies also provide information about the clinical and pathological disease characteristics that can be expected if a TSE crosses the species barrier, thus enabling animal health specialists to recognize such situations should they occur. Additional transmission studies in the natural host will focus on determining the modes of transmission and disease development in scrapie and CWD so that appropriate intervention strategies can be devised that will control the spread of these diseases. Studies, in collaboration with APHIS, are underway to determine the role of genetics in the susceptibility of elk to CWD. The scrapie, CWD and TME agents will be used in experiments to further assess the risk that these TSEs might pose to cattle, sheep, and swine. These experiments will also provide information and tissues that can be used to evaluate the effectiveness of current diagnostic protocols for animal TSEs. Cooperative research with the United Kingdom includes strain typing of CWD isolated from cases in the U.S.A. utilizing the mouse bioassay, use of recombinant prion proteins to develop or improve existing in vitro conversion strain-typing assays, use of immunohistochemistry to improve the standard pathology-oriented mouse strain-typing bioassay, and development of in vitro assays to predict infective titer of tissues derived from TSE-affected animals. Sheep scrapie studies are underway to determine influences of strain source and host genetics in disease progression. Current experiments are in progress to determine transmissibility of CWD from mule deer into cattle and

sheep. White-tailed deer have been inoculated with CWD from elk, mule deer and white-tailed deer to determine if there are strain differences in the agent that depend on host origin.

OVERALL PROJECT ACCOMPLISHMENTS:
Scientists on this project have demonstrated the transmissibility of scrapie to cattle by intracerebral inoculation and that the pathology of scrapie-affected cattle differed from BSE; *importantly*, cattle were shown to be resistant to challenge with scrapie by the oral route. Another significant accomplishment was the development and ongoing verification of the immunohistochemistry (IHC) test used by APHIS in the BSE surveillance program. A recent contribution of this project was the development of a method to conduct genotyping of the sheep prion gene using formalin-fixed and paraffin-embedded tissues as the DNA source material. Most recently, several of our research capabilities were utilized when project scientists contributed critical research in support of the 2003 BSE diagnosis. Western blot analyses and prion allele sequencing from the index case verified the accuracy of the APHIS IHC test and the species origin of the tissues as bovine. Current studies of CWD transmissibility to cattle indicate a relative resistance to infection and a pathology profile which differs from BSE. Another significant accomplishment was the demonstration that abnormal prions are not detectable by IHC in striated muscle tissues from animals experimentally inoculated with agents of TSEs. The current project has also demonstrated the utility of raccoons as a model for strain-typing TSEs; raccoons are very susceptible to TME, moderately so to scrapie, and resistant to CWD. In FY 2003 a mouse facility, dedicated for TSE strain typing of U.S. TSE isolates was built. This is a critical advance in our research capabilities to conduct the gold standard mouse bioassay for the differentiation of TSE strains ("strain-typing"). The mouse bioassay uses 4 inbred mouse strains with different prion protein genotypes. The new facility is essential for rearing these mice and is now operational. Mice from different PrP genotypes are being raised for future inoculation with defined U.S. scrapie and CWD isolates. Concurrently, in vitro strain typing efforts which rely on biochemical analysis of the pathological form of the prion protein (PrP^d) were started because the animal bioassay is relatively slow and expensive. This methodology involves determination of molecular mass and glycoform profile by Western Blot analysis. Preliminary results indicate CWD isolates from mule deer and white-tailed deer differ in molecular size when compared to CWD isolates from elk. Recently, completed work has determined that scrapie can be transmitted to elk and the resulting disease is indistinguishable from CWD in that species.

IMPACT:
Ongoing research verification of the IHC test used by APHIS has ensured the sensitivity and specificity of the test for BSE. Research support of the 2003 BSE case provided APHIS diagnostic pathologists with research verification of the IHC results and assurance the brain tissue was accurately identified as bovine in origin. A critical question for consumers is whether abnormal prion protein is present in meat, our research has shown that abnormal prion protein is not detectable by IHC in skeletal muscle tissues of animals experimentally inoculated with scrapie, CWD or TME. The demonstration that cattle and sheep are relatively resistant to experimental inoculation with CWD suggests that transmission of CWD to cattle or sheep in nature is not likely to occur easily. In addition, raccoons were shown to be a potential model for in vivo differentiation of the 3 animal prion diseases found in the United States (scrapie, CWD, and TME).

TECHNOLOGY TRANSFER:
The current and past cross-species-transmission TSE studies have generated a unique set of tissue reagents that have been shared with others for research and diagnostic purposes. This transfer of technology, along with the immunohistochemistry test developed by Dr. Miller at NADC (used around the world), has provided

diagnostic tools to many laboratories. Application of in vitro research techniques provided critical information to APHIS during the December 2003 diagnosis of BSE in a bovine from Washington State.

PUBLICATIONS:

Hamir, A.N., Clark, W.W., Sutton, D.L., Miller, J.M., Stack, M.J., Chaplin M.J., Jenny, A.L. Resistance of domestic cats to a US sheep scrapie agent by intracerebral route. Journal of Veterinary Diagnostic Investigation. 2002. v. 14. p. 444-445.

Hamir, A.N., Miller, J.M., Cutlip, R.C., Stack, M.J., Chaplin M.J., Jenny, A.L. Preliminary observations on the experimental transmission of scrapie to elk (Cervus elaphus nelsoni) by intracerebral inoculation. Veterinary Pathology. 2003. v. 40. p. 81-85.

Hamir, A.N., Miller, J.M., Stack, M.J., Chaplin, M.J. Failure to detect abnormal prion protein and scrapie-associated fibrils 6 wk after intracerebral inoculation of genetically susceptible sheep with scrapie agent. Canadian Journal of Veterinary Research. 2002. v. 66. p. 289-294.

Hamir, R. Cutlip, J. Miller, R. Kunkle, E. Williams, M. Stack, M. Chaplin, K. O'Rourke, J.A. Richt. 2004. Experimental cross-species transmission of chronic wasting disease to domestic livestock. *J. Vet. Diagn. Invest*, in press

Hamir AN, Miller JM, Cutlip RC, Stack MJ, Chaplin MJ, Jenny AL, Williams ES: Experimental inoculation of scrapie and chronic wasting disease agents to raccoons (Procyon lotor). Veterinary Record, 153: 121-123, 2003.

Hamir AN, Miller JM, Cutlip RC: Failure to detect prion protein by immunohistochemistry in striated muscle tissues of animals experimentally inoculated with agents of transmissible spongiform encephalopathy. Veterinary Pathology, (In press).

Hamir AN, Miller JM, Bartz JC, Stack MJ, Chaplin MJ: Transmission of transmissible mink encephalopathy (TME) to raccoons (Procyon lotor) by intracerebral inoculation. Journal of Veterinary Diagnostic Investigation, 15: ___ - ___, 2004 (In press).

Hamir AN, Miller JM, Cutlip RC, Kunkle RA, Jenny AL, Stack MJ, Chaplin MJ, Richt JA: Transmission of Sheep Scrapie to Elk (Cervus elaphus nelsoni) by Intracerebral Inoculation: Final Outcome of the Experiment. Journal of Veterinary Diagnostic Investigation, 15: ___ - ___, 2004 (In press).

Hamir AN, Kunkle RA, Richt JA, Miller JM, Cutlip RC, Jenny AL: Experimental transmission of sheep scrapie by intracerebral and oral routes to genetically susceptible Suffolk sheep in the United States. Journal of Veterinary Diagnostic Investigation, 2004. (Submitted).

---------- Additional publications by scientist in this project. ----------

Hamir, A.N., Miller, J.M., Sonn, R.J. Meningoencephalitis and pneumonia associated with Cryptococcus neoformans infection in a free-ranging elk in the USA. Veterinary Record. 2002. v. 151. p. 332-333.

Mueller, C.A., Richt, J.A., Meyermann, R., Deininger, M., Schluesener, H. 2002. Accumulation of EMAP II^+ microglial cells in brains of Borna virus infected rats. *Neurosci. Lett*. 339: 215-218

Hamir, A.N., Smith, B.B. Severe biliary hyperplasia associated with liver fluke infection in an adult alpaca. Veterinary Pathology. 2002. v. 39. p. 592-594.

Hamir, A.N., Timm, K.I. Nodular hyperplasia and cysts in thyroid glands of llamas (Lama glama) from north-west USA. Veterinary Record. 2003. v. 152. p. 507-508.

Palmer, M.V, Stoffregen, W.C., Rogers, D.G., Hamir, A.N., Richt, J.A., Pederson, D., Waters, W.R. 2004. West Nile Virus Infection in reindeer (Rangifer taratandus): report of 4 cases. *J. Vet. Diagn. Invest*, in press

Kiermayer, S., Kraus, I., Richt, J.A., Garten, W., Eickmann. M. 2002. Identification of the amino terminal subunit of the glycoprotein of Borna disease virus. *FEBS Lett.* 531:255-258.

Wolff, T., Unterstab, G., Heins, G., Richt, J.A., Kann, M. 2002. Characterization of an unusual importin alpha binding motif in the borna disease virus p10 protein that directs nuclear import. *J. Biol. Chem.* 277:12151-12157.

Dietzschold, B., Richt, J.A. (editors). 2002. Protective and Pathological Immune Responses in the CNS. *Curr. Top. Microbiol. Immunol*. 265: 1-267.

Hooper, D.C., Sauder, C., Scott, G.S., Dietzschold, B., Richt, J.A. 2002. Immunopathology and immunoprotection in CNS virus infections: mechanisms of virus clearance from the CNS. In: Dietzschold, B., Richt, J.A. (eds). Protective and Pathological Immune Responses in the CNS. *Curr. Top. Microbiol. Immunol*. 265: 163-182.

Dubey JP, Hamir AN, Topper MJ: Sarcocystis mephitisi N. Sp. (Protozoa: Sarcocystidae), Sarcocystis neurona-like and Toxoplasma-like infections in striped skunk (Mephitis mephitis). Journal of Parasitology. 88: 113-117, 2002.

Hamir AN, Olsen S, Rupprecht CE: Granulomatous orchitis associated with Histoplasma-like organisms in porcupines (Erethizon dorsatum). Veterinary Record 150: 251-252, 2002.

Hamir AN, Miller JM, Stack MJ, Chaplin MJ, Cutlip RC: Neuroaxonal Dystrophy in Raccoons (Procyon lotor) from Iowa. Journal of Veterinary Diagnostic Investigation. 14: 175-178, 2002.

Dubey JP, Hamir AN: Experimental toxoplasmosis in budgerigars (Melopsittacus undulates). Journal of Parasitology. 88: 514 - 519, 2002.

Heidari M, Hamir AN, Cutlip RC, Brogden KA: Antimicrobial anionic peptide binds in vivo to Mannheinia (Pasteurella) haemolytica attached to ovine alveolar epithelium. The
International Journal of Antimicrobial Agents. 20: 69-72, 2002.

Hamir AN, Stasko J, Rupprecht CE: The observation of Helicobacter-like organisms in gastric mucosa of grey foxes (Urocyon cinereoargenteus) and bobcats (Lynx rufus). Canadian Journal of Veterinary Research. (In press).

Hamir AN, Sonn RJ, Franklin S, Wesley IV: Enteritis with intestinal intussusception associated with Campylobacter jejuni infection in a raccoon (Procyon lotor) kit and isolation of C. jejuni and Arcobacter spp. from adult raccoons. The Veterinary Record. (In press).

Sacco, R.E., Nestor, K.E., and Kunkle, R.A. Genetic variation in response of turkeys to experimental infection with Bordetella avium. Avian Dis. 44:197-200. 2000.

Kalfa, V.C., Jia, H.P., Kunkle, R.A., McCray, P.B. Jr., Tack, B.F., Brogden, K.A. Congeners of SMAP29 kill ovine pathogens and induce ultrastructural damage in bacterial cells. Antimicorb Agnts Chemother. Nov;45(11):3256-61. 2001.

Brockmeier, S.L., Register, K.B., Magyar, T., Lax, A.J., Pullinger, G.D., Kunkle, R.A. Role of the dermonecrotic toxin of Bordetella bronchiseptica in the pathogenesis of respiratory disease in swine. Infect Immun. Feb:70(2):481-90. 2002.

Kunkle, R.A. Fungal Infections. *In*: Diseases of Poultry, 11th Ed. Barnes, H.J., Fadly, H.M., Glisson, J.R., McDougold, L.R., Swayne, D.E., eds. Iowa State Press, Ames, Iowa, U.S.A., pp. 883-896. 2003.

Kunkle, R.A. (contributor), Fowl Cholera (Avian Pasteurellosis). *In*: OIE Manual of Standards for Diagnostic Tests and Vaccines, List A and B diseases of mammals, birds and bees. Office International des Epizooties, Paris, France. In Press.

Respiratory Diseases of Livestock

CRIS Title: Respiratory Diseases of Poultry
CRIS Number:: 3625-32000-047
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PROJECT OBJECTIVES:
Fowl cholera (caused by *Pasteurella multocida*) and bordetellosis (caused by *Bordetella avium*) are bacterial diseases responsible for major economic losses to the poultry industry, especially the turkey industry. Bordetellosis usually affects young turkeys, 1 day to 6 weeks of age, while fowl cholera usually affects turkeys at 8 weeks of age and older. Often turkeys may experience both diseases, i.e., bordetellosis followed by fowl cholera. Live-attenuated vaccines against fowl cholera often fail to protect turkeys after the birds have experienced bordetellosis, and killed vaccines against fowl cholera do not protect poultry against different types of *P. multocida*. The bacterial components and host conditions responsible for these actions have not been identified. The components of *P. multocida* that produce immunity to the same and different types of the bacteria are being purified and evaluated for use as protective vaccines against fowl cholera and for use in developing tests of immunity in vaccinated flocks. The components of *B. avium* responsible for immune suppression are being identified and their mechanisms of action on the turkey and its immune system are being characterized.

OVERALL PROJECT ACCOMPLISHMENTS:
Hyaluronic acid, a component of host tissues and the capsule of *P. multocida*, was found to be responsible for attachment of the bacteria to turkey air sac macrophages, but not to blood monocytes. Growth of monocytes in tissue culture resulted in upregulation of a glycoprotein receptor. Because bacterial invasion of the host initially involves attachment to a host receptor, this glycoprotein may be involved in invasion by *P. multocida*. Methods or materials, which inhibit bacterial attachment to this receptor, may provide a means to protect against fowl cholera.

Turkeys infected with *B. avium* respond poorly to vaccination with live-attenuated vaccines against fowl cholera. Research has shown that *B. avium* infection did not result in a poor immune response to vaccination when bacterins are used to control fowl cholera. These findings imply that strategies can be developed whereby immunization of breeder hens to *B. avium* and vaccination of their offspring with *P. multocida* bacterins will control fowl cholera in turkeys when vaccination with live-attenuated vaccines of *P. multocida* is not feasible.

The antigen in *P. multocida* that produces protection against different serotypes of the bacteria was identified and purified. This antigen is most likely responsible for the protection against different serotypes of *P. multocida* conferred by live-attenuated vaccines. With identification and further characterization of the protective determinant, it may be possible to develop reagents and tests to predict flock immunity produced by live-attenuated vaccines.

A method for DNA fingerprinting *R. anatipestifer* was developed. This molecular method can be used to determine the epidemiology of *R. anatipestifer* in turkeys and ducks.

A method for DNA fingerprinting *B. avium* was developed. This molecular method can differentiate *B. avium* from the non-pathogenic *B. hinzii*, which often occurs in turkeys and can be used to determine the epidemiology of *B. avium*.

Monoclonal antibodies to the type specific antigen of serotypes 1, 2, and 3 *P. multocida* were developed and will be used to develop new and simpler tests for serologic typing of *P. multocida*.

Little is known about the transmission or prevalence of *B. avium* in wild birds or whether wild birds may act as a reservoir for domesticated poultry. We surveyed 237 birds, representing 61 wild bird species, for evidence of past or present *B. avium* infection. Approximately 42% of the birds sampled, representing 41 species, were identified as having been infected with *B. avium*. DNA analysis of *B. avium* isolates from wild birds showed that at least some were indistinguishable from clinical isolates from domesticated turkeys. These results suggest that transmission of *B. avium* from free-living avian populations may be possible and should be of concern to producers.

Two genetic lines of turkeys were infected with *B. avium* to determine whether there might be genetic variation in resistance to bordetellosis. No differences were found in the time of onset, incidence, or severity of clinical signs of bordetellosis between the two lines of poults infected with *B. avium*. However, there was a significant depression (12%) in body weight of one line of poults selected for rapid growth rate. This study is the first to show genetic variation in response of poults to experimental infection with *B. avium* and suggests that disease resistance to bordetellosis may be further improved by genetic selection.

With researchers at the UCLA School of Medicine, two new avian epithelial antimicrobial peptides, Gallinacin3 and gallopavin1, were identified in tissues from chickens and turkeys, respectively. Expression of Gallinacin3 was especially prominent in the tongue, bursa of *Fabricius*, and trachea of chickens. Tracheal expression of Gallinacin3 was significantly increased following infection with *Hemophilus paragallinarum*, whereas its expression in tongue, oesophagus, and bursa of *Fabricius* was unaffected. These peptides may be important in the host innate immune defense mechanism to respiratory tract infection.

With researchers at Ohio State University, Beltsville Small White turkeys were found to represent a unique source of variation in major histocompatibility complex (MHC) class II genes and could be useful for immunological experiments that require syngeneic (MHC-matched) cells. The MHC is a chromosomal region containing a family of genes that encode a group of proteins involved in regulating a number of cellular processes of importance to the immune response.

The research that was accomplished will assist in efforts to use the *Pasteurella* lipoprotein B protein for cloning, expressing and utilization in challenge studies for development of a new vaccine for fowl cholera. This progress report addresses Objective 1 of the research plan to determine the mechanisms of pathogenesis of *P. multocida*.

IMPACT:
The work described to date reflects activities that were instrumental to developing new objectives outlined in the recent OSQR Project Plan (submitted for review 9/5/03). The new objectives focus on bacterial respiratory diseases of poultry and are as follows: (1) Define virulence mechanisms of *Ornithobacterium rhinotracheale* (ORT) infection in poultry in order to optimize control strategies and diagnostic methodologies. (2) Determine the mechanisms of pathogenesis of *Bordetella avium* associated with tracheal colonization and immunosuppression in turkeys. (3) Identify and characterize virulence factors and or immunogens of *Pasteurella multocida* related to colonization and the development of immunity in poultry. The impact of the work has created the foundation to (1) develop DNA-based diagnostic tests for accurate bacterial identification and strain differentiation among isolates; (2) utilize mutant strains of bacteria to examine the pathogenesis related to toxin production and examine the genes involved in colonization; (3) express outer membrane proteins of the bacteria which could provide heterologous immunity to multiple serotypes and become effective vaccine candidates. This information will be useful in defining new methods for inhibiting infection in poultry.

TECHNOLOGY TRANSFER:
Conducted serotyping analysis of *P. multocida* strains for diagnostic laboratories and industry.

Conducted DNA fingerprinting on *R. anatipestifer* for The Michigan State University Diagnostic laboratory to determine vaccine efficacy.

Provided cultures of *P. multocida* and *H. gallinarum* to biologic companies, university laboratories and the NVI (Bucharest, Romania) for research, diagnostics and vaccine development.

Provided information pertaining to nucleic acid-based detection methods for *Bordetella* and *Pasteurella* to an international online news and information provider specific to animal health industries.

PUBLICATIONS:

Tabatabai, L.B.**,** Zehr, E.S. Identification of five outer membrane-associated proteins among cross-protective factor proteins of *Pasteurella multocida.* Infection and Immunity. 2004. 72:1195-1198.

Register, K.B., Sacco, R.E., Nordholm, G.E. Comparison of ribotyping and restriction enzyme analysis for inter- and intraspecies discrimination of *Bordetella avium* and *Bordetella hinzii*. Journal of Clinical Microbiology. 2003. v. 41(4). p. 1512-1519.

Andersen, A.A., Vanrompay, D. Avian chlamydiosis (*Psittacosis*, *Ornithosis*). Saif, Y.M., Barnes, H.J., Fadly, A.M., Glisson, J.R., McDougald, L. R., Swayne, D.E., editors. Iowa State Press, Ames, IA. Diseases of Poultry, 11th Edition. 2003. Chapter 25, p. 863-879.

Kunkle, R.A. Fungal infections. Saif, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M., McDougald, L.R., Swayne, D.E., editors. Iowa State Press, Ames, IA. Diseases of Poultry, 11th Edition. 2003. p. 883-896.

Raffel, T.R., Register, K.B., Marks, S.A., Temple, L. 2002. Prevalence of *Bordetella avium* infection in selected wild and domesticated birds in the Eastern USA. Journal of Wildlife Diseases. 2002. 38:40-46.

Murphy, E.R., Sacco, R.E., Dickerson, A., Metzger, D.J., Hu, Y., Orndorff, P.E., Connell, T.D. Bhur, a virulence-associated outer membrane protein of *Bordetella avium*, is required for the acquisition of iron from heme and hemoproteins. Infection and Immunity. 2002. 70:5390-5403.

Zhao, C., Nguyen, T., Liu, L., Sacco, R.E., Brogden, K.A., Lehrer, R.I. Gallinacin-3, an inducible epithelial b-defensin of the chicken. Infection and Immunity. 2001. 69:2684-2691.

Sacco, R.E., Rimler, R.B., Ye, X., Nestor, K.E. Identification of new major histocompatibility complex class II restriction fragment length polymorphisms in a closed experimental line of turkeys. Poultry Science. 2001. 80:1109-1111.

Sacco, R.E., Register, K.B., Nordholm, G.E. Restriction enzyme analysis and ribotyping distinguish *Bordetella avium* and *Bordetella hinzii* isolates. Epidemiology of Infection. 2000. 124:83-90.

Sacco, R.E., Nestor, K.E., Kunkle, R.A. Genetic variation in response of turkeys to *Bordetella avium*. Avian Diseases. 2000. 44:197-200.

Respiratory Diseases of Livestock

CRIS Title: Control of Porcine Respiratory Diseases of Complex Etiology
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PROJECT OBJECTIVES:
Respiratory disease in swine is complex due to interactions between different kinds of bacteria or between viruses and bacteria, including *Bordetella bronchiseptica*, *Pasteurella multocida*, and *Haemophilus parasuis*. They often act as secondary lung pathogens following viral infections. Vaccines containing killed *B. bronchiseptica* and *P. multocida* do provide at least some benefit in reducing the economic impact of these diseases. However, surveys reveal that the majority of U.S. producers find the efficacy of these vaccines to be low. A major impediment to the development of improved vaccines is a gap in our understanding of the identity and function of those products of *B. bronchiseptica*, *P. multocida*, and *H. parasuis* that permit the bacteria to colonize swine and that cause disease. Understanding bacterial and host mechanisms involved in the pathogenesis of porcine respiratory disease will permit more effective preventive measures and control strategies to be developed. Models for respiratory diseases in swine caused by *B. bronchiseptica* and *P. multocida* alone, together, and in combination with *H. parasuis* and viruses such as Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) are being developed. Products of *B. bronchiseptica*, *P. multocida*, or *H. parasuis* suspected to have a role in development of disease or inducing immune protection are being evaluated in laboratory culture systems or in animal models to determine their actual functions. Additionally, both *B. bronchiseptica* and *P. multocida* are difficult to detect using standard laboratory procedures and, as a result, their impact in disease is probably underestimated. New, more sensitive nucleic acid-based tests are being developed for their identification and for use in distinguishing different strains.

OVERALL PROJECT ACCOMPLISHMENTS:
DNA vaccines designed to induce cell-mediated and/or antibody responses to the *P. multocida* toxin (PMT) have been constructed. The vaccines consist of 2 slightly different eukaryotic expression vectors into which we have cloned either a truncated PMT gene that retains all known immunogenic epitopes or the full-length PMT gene, but with a single point mutation that results in genetic detoxification. Immunization of mice with the recombinant plasmids demonstrated no toxic effects from any of the constructs. However, only one of the constructs containing the entire PMT gene appears to be immunogenic in mice.

We identified two colonization factors for *H. parasuis*, a P-5 protein and a P-2 protein based on homologies to *H. influenzae* proteins. Using monoclonal antibodies we determined that the avirulent reference strains contain the P-2 protein as a 55 kDa protein with the exception of serovar 7 and that all virulent strains contain the P-2 protein as a 48 kDa protein with the exception of serovar 4. The P-5 protein was purified, characterized and the gene for the P-5 protein has been cloned. Field strains will be tested for the presence of P-2 and the P-5 proteins.

Whether or not aerosol spread of respiratory pathogens occurs is of importance for determining methods for breaking the transmission cycle. We determined that aerosol transmission of both PRRSV and B.

bronchiseptica occurs, at least over short distances. Thus animals do not necessarily have to be in direct contact to transmit these diseases among the herd.

Although vaccines for *B. bronchiseptica* have been widely utilized for many years, improvements are needed since a majority of swine veterinary specialists report dissatisfaction with their protective efficacy. The protein pertactin that is produced by *B. bronchiseptica* has been implicated as an adhesin and protective immunogen in swine. The DNA and predicted amino acid sequences of the pertactin repeat regions from 81 recent field isolates and 5 vaccine strains from the United States were determined, and 4 unique pertactin types were identified. Interestingly, only 2 vaccines possess the most commonly identified pertactin type found in the swine isolates. If immune protection to *B. bronchiseptica* correlates with the presence of pertactin, then vaccine efficacy could be improved by including pertactin types matching those commonly circulating in the field.

The protein filamentous hemagglutinin (FHA) may also be an important protective antigen for *Bordetella* species. Analysis of the FHA genes from different *B. bronchiseptica* isolates revealed differences in the DNA sequence and protein structure. Heterogeneity in this gene was not previously known to exist. It may be possible to improve the efficacy of swine vaccines by ensuring they contain forms of FHA identical to those found in swine isolates.

P. multocida toxin (PMT), known to cause loss of bone density, also causes alterations in the cells involved in bone remodelling. B lymphocytes in the bone marrow may interact with these bone cells during their development. We have developed an in vitro porcine bone marrow culture system in order to examine whether changes in bone cells induced by treatment with PMT influence the development of B cells in bone marrow. Modulation of B cell development could influence the host response to secondary infections.

Haemophilus parasuis is a re-emerging pathogen of high-health status swine. We have performed a cluster analysis of the one-dimensional gel protein profiles of all serovars. Fifteen serovars have been identified of which six are highly virulent (cause death), four are less virulent (cause no death), and five are completely avirulent. The results revealed that there are two main groups consisting of the highly virulent and less virulent strains and six sub groups consisting of similar protein profiles.

Secondary pathogens such as *P. multocida* and *H. parasuis* have difficulty establishing infection on their own. We have shown that prior infection with *B. bronchiseptica* enhances colonization of both *P. multocida* and *H. parasuis* in the swine upper respiratory tract. Thus, reduction of *B. bronchiseptica* infection should decrease the incidence of secondary respiratory infections.

Infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is thought to predispose to pneumonia with bacterial pathogens. We have shown that PRRSV predisposes to bronchopneumonia with *B. bronchiseptica* but not *P. multocida*. Unlike *B. bronchiseptica*, *P. multocida* does not colonize the respiratory tract well on its own. Enhancing the colonization of *P. multocida* through prior infection with *B. bronchiseptica* resulted in increased incidence of bronchopneumonia with *P. multocida* when the pigs were coinfected with PRRSV. Producers should minimize this combination of organisms in their herds since more severe disease, and possibly further infection with opportunistic bacteria, may occur in co-infected herds.

A *B. bronchiseptica* mutant unable to produce the dermonecrotic toxin, believed to be its primary virulence

factor, was produced and subsequently evaluated in swine. The results demonstrated that the toxin is important for much of the disease manifestations, but that other virulence factors appear responsible for establishing infection as the mutant was still able to colonize well. Subsequently, experiments were conducted to determine whether colonization despite the attenuation of disease would be able to predispose to secondary infections. It was found that infection with the dermonecrotic toxin mutant of *B. bronchiseptica* was still able to enhance colonization with *P. multocida* resulting in lesions of atrophic rhinitis.

Swine isolates of *B. bronchiseptica* were found to be resistant to killing by sera. Although the genetic locus conferring serum resistance for other *Bordetella* species (brkAB) was demonstrated to be present in *B. bronchiseptica*, construction and analysis of a mutant strain revealed that some other mechanism must underlie serum resistance in this species. Therefore, the products of the brkAB genes are not likely to be beneficial as components of a vaccine.

Ribotyping was developed as a molecular method to discriminate among strains of *B. bronchiseptica* and was found to be useful as a tool for investigating outbreaks. Restriction enzyme analysis was developed as a molecular method to distinguish among *Bordetella bronchiseptica* isolates.

A sensitive and specific diagnostic test was developed to simultaneously identify *B. bronchiseptica* and toxigenic *P. multocida* in cultures from swine with respiratory disease. The test should eliminate many false negative results obtained with conventional methods currently in use in clinical laboratories.

A *B. bronchiseptica* mutant deficient in a major pathway for iron acquisition was constructed and evaluated in swine. The mutation significantly decreased the bacteria's virulence, though not sufficiently to be useful as an attenuated vaccine. However, the results demonstrated that disruption of iron acquisition may provide a means to control infection.

B. bronchiseptica was found to be capable of invading and surviving within swine alveolar macrophages, which are a primary host defence mechanism in the lung. Furthermore, *B. bronchiseptica* was demonstrated to be cytotoxic for these cells. These observations may explain, in part, the ability of this organism to establish infection in an otherwise healthy animal and to predispose infected swine to secondary respiratory pathogens.

We demonstrated in vivo and in vitro that greater numbers of bacteria adhere to cells when *B. bronchiseptica* is grown at room temperature compared to body temperature, indicating a potential environmental phase in transmission. *B. bronchiseptica* was found to attach to swine ciliated nasal epithelial cells through a novel mechanism that has not been previously described. The attachment factor(s) involved is not produced under conditions typically used for the growth of bacteria included in bacterin vaccines. Vaccine efficacy may be improved by changes in growth conditions that would permit production of this attachment factor.

We have established a protein expression map of each of the 15 serovars of *H. parasuis* under an iron-sufficient growth condition. This map in addition to others which will be prepared under other nutrient limitation or environmental stress will allow us to identify unique proteins of the virulent reference strains compared to the avirulent reference strains.

We identified two unique and two common proteins by comparing the composites of two virulent reference strains and two avirulent reference strains grown under iron-sufficient growth conditions. The four proteins

were characterized by mass spectrometry. One of the unique proteins is a homolog of the *H. influenzae* "protective antigen." The gene encoding this protein will be cloned, expressed, and purified for preliminary vaccine trials in Caesarean-derived, colostrum-deprived (CDCD) nursery pigs.

We identified a virulence factor and colonization factor of *H. parasuis* by homology to *H. influenzae* using *H. influenza* primers and PCR. This colonization factor was cloned and expressed in *E. coli*. The recombinant adhesion protein P5 will be purified by affinity chromatography and tested in CDCD pigs for its ability to prevent colonization of the upper and lower respiratory tract.

IMPACT:

The work addresses these objectives in our OSQR Project Plan: to determine the ability of these organisms and their products to act in concert with other respiratory pathogens to exacerbate disease; to identify, through genomic and proteomic analysis, changes in gene expression of the host and infectious agents during the infectious process; and to evaluate intervention strategies to control porcine respiratory disease, including vaccination. The accumulating amount of evidence indicates that *B. bronchiseptica* is important in predisposing to a broad spectrum of respiratory diseases in swine and control of this pathogen will be important for overall respiratory health of swine. Identification of proteins unique to virulent strains of *H. parasuis* will impact vaccine development and diagnostics.

TECHNOLOGY TRANSFER:

Provide consultation to veterinary biologics companies, swine production management companies and producers.

Have material transfer agreements with several veterinary biologics companies.

Have CRADA 58-3K95-2-921 with BRDC (Effects of PRRSV infection on gene expression in swine).

Have provided ISU diagnostic lab with *Pasteurella multocida* toxin testing via colony lift assay and PCR methods and transferred the PCR technology for them to use.

Used ribotyping to investigate the epidemiology of outbreaks of *Bordetella bronchiseptica* for several swine production management companies.

PUBLICATIONS:

Brockmeier, S.L. Prior infection with *Bordetella bronchiseptica* increases colonization with *Haemophilus parasuis* in swine. Veterinary Microbiology. 2004. 99:75-78.

Brockmeier, S.L., Lager, K.M. Tissue distribution of porcine reproductive and respiratory syndrome virus (PRRSV) in finisher age boars and gilts at various times post infection. Proceedings of the18th International Pig Veterinary Society Congress, Hamburg, Germany. 2004.

Staveley, C.M., Register, K.B., Miller, M.A., Brockmeier, S.L., Jessup, D.A., Jang, S. Molecular and antigenic characterization of *Bordetella bronchiseptica* isolated from a wild southern sea otter (*Enhydra lutris nereis*) with severe suppurative bronchopneumonia. Journal of Veterinary Diagnostic Investigation. 2003. 15:70-74.

Brockmeier, S.L., Register, S.L. A *Bordetella bronchiseptica* DNT mutant predisposes pigs to infection with toxigenic *Pasteurella multocida*. ASM, Washington D.C. 2003.

Brockmeier, S.L. Atrophic rhinitis and beyond. Pig Progress. Special Swine Respiratory Diseases Issue. 2003.

Register, K.B. DNA-based tools for identification of *Pasteurella multocida* and *Bordetella bronchiseptica*. Feedinfo News Service. feedinfo.com. posted on 12-12-03 under Scientific Reviews. 2003.

Foster, G., Register, K.B., Patterson, I.A.P., and Sacco, R.E. Re-emergence of *Bordetella bronchiseptica* during the recent seal morbillivirus outbreak. Proceedings of the Scottish Agricultural College of Veterinary Sciences Conference. 2003. p. 21.

Loving, C.L., Sacco, R.E. The effects of *Pasteurella multocida* toxin (PMT) on early murine B lineage cells. Autumn Immunology Conference, November, 2003, Chicago, IL.

Brockmeier, S.L., Register, K.B., Magyar, T., Lax, A., Pullinger, G., Kunkle, R.A. Role of the dermonecrotic toxin of *Bordetella bronchiseptica* in the pathogenesis of the respiratory tract of swine. Infection and Immunity. 2002. 70:481-490.

Brockmeier, S.L., Lager, K.M. Experimental airborne transmission of porcine reproductive and respiratory syndrome virus and *Bordetella bronchiseptica*. Veterinary Microbiology. 2002. 89:267.

Brockmeier, S.L., Lager, K.M., Palmer, M.V. Interactions among porcine reproductive and respiratory syndrome virus (PRRSV), *Bordetella bronchiseptica* and *Haemophilus parasuis* in swine respiratory disease. Proceedings of the 17th International Pig Veterinary Society Congress, Ames, IA. 2002.

Janutenaite, J., Lager, K., Stabel, T., Pesch, B., Brockmeier, S. Evaluation of immune response induced by attenuated and wild-type PRRS virus. Proceedings of the17th International Pig Veterinary Society Congress, Ames, IA. 2002.

Lager, K.M., Brockmeier, S.L. A review of area spread and summary of recent studies. Allen D. Leman Swine Conference, Minneapolis, MN. 2002.

Brockmeier, S.L., Halbur, P.G., Thacker, E.L. Porcine respiratory disease complex. In Polymicrobial Diseases, K.A. Brogden and J. Guthmiller Editors. ASM Press. 2002.

Register, K.B. Comparative sequence analysis of *Bordetella bronchiseptica* pertactin gene repeat regions. Proceedings of the 17th International Pig Veterinary Society Congress. 2002. p. 342.

Register, K.B. Molecular tools for identification of *Bordetella bronchiseptica*. Respiratory Diseases VI. Pig Progress. 2002. p.20-21.

Tabatabai, L.B. Using proteomics in diagnostics. IVD Technology. 2002. 8(7):37-48.

Brockmeier, S.L., Palmer, M.V., Bolin, S.R., Rimler, R.B. Effects of intranasal inoculation with *Bordetella bronchiseptica*, porcine reproductive and respiratory syndrome virus, or a combination of both organisms on subsequent infection with *Pasteurella multocida* in pigs. American Journal of Veterinary Research. 2001. 62:521-525.

Register, K.B., Ducey, T., Brockmeier, S.L., Dyer, D.W. Reduced virulence of a *Bordetella bronchiseptica* siderophore mutant in neonatal swine. Infection and Immunity. 2001. 69:2137-2143.

Brockmeier, S.L. *Bordetella* and *Pasteurella*: Their role in PRDC and re-emergence of atrophic rhinitis. Proceedings of the Iowa Veterinary Medical Association, Ames, Iowa. 2001.

Brockmeier, S.L., Palmer, M.V. Prior infection with *Bordetella bronchiseptica* increases colonization with *Haemophilus parasuis* in swine. Proceedings of the 82nd Conference of Research Workers in Animal Diseases. St. Louis, Missouri. 2001.

Janutenaite, J., Lager, K., Stabel, T., Pesch, B., Brockmeier, S. Comparison of cellular immune response induced by attenuated and wild-type PRRS virus. Proceedings of the 82nd Conference of Research Workers in Animal Diseases, St. Louis, Missouri. 2001.

Register, K.B. Genetic polymorphism and its implications in *Bordetella bronchiseptica*. Respiratory Diseases V. Pig Progress. 2001. 20-21.

Register, K.B. Novel genetic and phenotypic heterogeneity in *Bordetella bronchiseptica* pertactin. Infection and Immunity. 2001. 69:1917-1921.

Lenart, J., Andersen, A.A., Rockey, D.D. Growth and development of tetracycline resistant *Chlamydia suis*. Antimicrobial Agents and Chemotherapy. 2001. 45:2198-2203.

Brockmeier, S.L., Register, K.B. Effect of temperature modulation of *Bordetella bronchiseptica* on adhesion, intracellular survival and cytotoxicity for swine alveolar macrophages. Veterinary Microbiology. 2000. 73:1-12.

Brockmeier, S.L., Palmer, M.V., Bolin, S.R. Effects of intranasal inoculation of porcine reproductive and respiratory syndrome virus, *Bordetella bronchiseptica*, alone or in combination, in pigs. American Journal of Veterinary Research. 2000. 61:892-899.

Brockmeier, S.L., Register, K.B., Kunkle, R.A. Evaluation of the virulence of a dermonecrotic toxin mutant of *Bordetella bronchiseptica* in swine. Proceedings of the 16th International Pig Veterinary Society Congress, Melbourne, Australia. 2000. p.480.

Brockmeier, S.L., Palmer, M.V., Bolin, S.R., Rimler, R.B., Lager, K.M. Interactions among porcine reproductive and respiratory syndrome virus (PRRSV), *Bordetella bronchiseptica* and *Pasteurella multocida* in swine respiratory disease. Proceedings of the 16th International Pig Veterinary Society Congress, Melbourne, Australia. 2000.

Register, K.B. RFLP and sequence analysis reveals heterogeneity among the pertactin genes of *Bordetella bronchiseptica* vaccine and field strains. Proceedings of the 16th International Pig Veterinary Society Congress. 2000. p. 481.

Sacco, R.E., Register, K.B., Nordholm, G.E. Restriction endonuclease analysis discriminates *Bordetella bronchiseptica* isolates. Journal of Clinical Microbiology. 2000. 38:4387-4393.

Register, K.B., Sacco, R.E., and Foster, G. Ribotyping and restriction endonuclease analysis reveal a novel clone of *Bordetella bronchiseptica* in seals. Journal of Veterinary Diagnostic Investigation. 2000. 12:535-540.

Register, K.B. *Bordetella bronchiseptica*--A New Look at an Old Pathogen. Respiratory Diseases IV Pig Progress. 2000. p. 28-29.

Register, K.B. *Bordetella bronchiseptica*--an underestimated threat? Pig Progress. 2000. 16:10-11.

Waters, W.R., Hontecillas, R., Sacco, R.E., Zuckermann, F.A., Harkins, K.R., Bassaganya-Riera, J., Wannemuehler, M.J. Antigen-specific proliferation of porcine CD8αα cells to an extracellular bacterial pathogen. Immunology. 2000. 101:333-341.

Rogers, D.G., Andersen, A.A. Intestinal lesions caused by a strain of *Chlamydia suis* in weanling pigs infected at 21 days of age. Journal of Veterinary Diagnostic Investigation. 2000. 12:233-239.

Respiratory Diseases of Livestock

Project Title: Controlling Losses from Respiratory Disease in Cattle, Sheep, and Goats
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PROJECT OBJECTIVES:

Respiratory tract diseases are the leading cause of loss from disease in the cattle, sheep, and goat industries. Annual loss in the United States is estimated to exceed one billion dollars for cattle alone. Viral agents are well recognized as primary pathogens and even uncomplicated infections can cause substantial economic loss. Infectious Bovine Rhinotracheitis Virus (IBRV), Respiratory Syncytial Virus (RSV), Parainfluenza Type 3 Virus (PI3V), and Bovine Viral Diarrhea Virus (BVDV) are generally considered the most economically important viruses against which exist numerous commercially-available multivalent vaccines. Yet the magnitude and complexity of the problem still exists. A portion of this problem is attributable to Respiratory Coronavirus and Adenoviruses, which have not been extensively evaluated. Respiratory viruses also predispose animals to costly bacterial pneumonia caused by *Mannheimia haemolytica*, *Pasteurella multocida*, *Haemophilus somnus*, and *Mycoplasma bovis* which commonly affect beef cattle. *P. multocida* and *M. haemolytica* commonly affect dairy cattle. These bacteria remain the major cause of loss from mortality, reduced feed efficiency, treatment costs, and reduced product quality. Commercial vaccines are available for bacterial respiratory disease, but their efficacy in the field is questionable. An underlying problem is the current beef cattle marketing system, which does not allow timely vaccination before the peak period of disease occurring soon after shipping. Antibiotic usage at this time is prevalent, costly, but beneficial. Vaccination in sheep and goats is complicated by the broad number of *M. haemolytica* serovars against which protection is necessary. The role of Adenovirus in causing or contributing to respiratory disease is being evaluated using serologic and virus isolation methods. The molecular mechanisms of respiratory tract colonization and disease progression are being elucidated by construction and testing of non-polar bacterial mutants. New modified-live vaccine candidates for rapid natural induction of disease resistance are being constructed and evaluated. Finally, innate mechanisms of respiratory tract resistance to bacterial infection are being characterized as a new potential point for disease intervention.

OVERALL PROJECT ACCOMPLISHMENTS:

To better understand disease progression and to produce better vaccines, techniques were developed to genetically manipulate *M. haemolytica*, *P. multocida*, and *H. somnus*. Many of these techniques now are patented, and four organisms so produced are currently licensed for use in veterinary vaccines. A combination *M. haemolytica*/*P. multocida* single-dose oral vaccine was formulated to address the need for rapid immunity and reduction of injection-site reactions. Evaluation in laboratory and field experiments show that the vaccine reduces nasal colonization by *M. haemolytica*, pneumonic lung lesions, and mortality from respiratory disease. Vaccination with the new vaccine significantly improves the performance of calves in the feedlot, a benefit not reported for any currently available respiratory disease vaccine. A live vaccine strain was constructed for protection of wild Bighorn sheep, which may prove effective following application on hay in the winter. These studies involve the Pathogen Detection and Diagnostics, Mechanisms of Disease, Epidemiology of Disease, and Strategies to Control Infectious and Non-Infectious Disease Components of the Animal Health Program.

High-quality reagents to characterize adenoviruses from ruminants have been lacking in the United States. Plaque-purified clones and antibodies for prototype cattle, sheep, and goat adenoviruses were produced and tested for purity. The adenoviruses and antiserum are being used as reference reagents to serotype newly-isolated adenoviruses from ruminants and to determine the prevalence of this group of viruses in the ruminant population. Two new species of adenovirus were isolated and characterized from goats, and one was isolated from deer. Two species of bovine adenovirus were isolated and characterized for the first time from U.S. cattle. Bovine adenovirus 10 has been shown to produce clinical disease and lesions in calves. Knowledge gained will allow for the determination of importance of specific serotypes of adenovirus in the respiratory disease complex so that methods of control and diagnosis can be developed. These studies involve the Pathogen Detection and Diagnostics, Mechanisms of Disease, Epidemiology of Disease, and Strategies to Control Infectious and Non-Infectious Disease Components of the Animal Health Program.

Not all animals exposed to *M. haemolytica* develop pneumonia, and these differences may be related to innate pulmonary defense mechanisms. Three antimicrobial anionic peptides were isolated from pulmonary surfactant of cattle and sheep. These peptides were small (721.6 – 823.7 Da), hydrophilic, and contained repeats of aspartic acid residues. Minimal inhibitory concentrations of anionic peptides and similar analogs were comparable to those of other vertebrate antimicrobial peptides. Other antimicrobial peptides have also been found in ruminants that are also efficacious. These include proline-rich antimicrobial peptides from sheep and goat leukocytes and beta defensins. Further study of these innate immune mechanisms may allow the elucidation of new potential points for disease intervention. These studies involve the Animal Immunology, Genetic Resistance to Disease, and Strategies to Control Infectious and Non-Infectious Disease Components of the Animal Health Program.

IMPACT:
Novel delivery of new bacterial respiratory disease vaccines has reduced nasal colonization, reduced re-treatment morbidity costs, reduced mortality losses, and repeatedly improved the performance of cattle placed into feedlots, addressing OSQR Objectives 1 and 2. Commercial veterinary biologics manufacturers are interested in marketing vaccines based on this technology. Improvements in diagnostic capabilities for Adenovirus have allowed us to identify type 7 as a prevalent problem among cattle. These improvements directly address OSQR Objective 3 as we seek to determine the importance of Adenovirus in bovine respiratory disease.

TECHNOLOGY TRANSFER:
Presented research progress and updates regarding modified-live mucosal vaccination of cattle against bacterial bovine respiratory disease at National Cattlemen's Beef Association Annual Conventions and United States Animal Health Association meetings.

Invited to numerous veterinary biologics firms to present research progress on construction and use of modified-live vaccine organisms.

Transferred plasmid constructs, recombinant organisms, and technical assistance to veterinary biologics firms and university projects, including one studying human gingivitis and two working with bighorn sheep.

Consulted and assisted commercial veterinary biologics firms regarding design, construction, and evaluation of vaccine candidates.

Provided extensive field support for multiple university projects examining the impact of respiratory coronavirus, bovine viral diarrhea, and specific vaccination, on calves under typical field conditions.

Provided *Mannheimia* diagnostic assistance, reagents, and bacterial strains to scientists in domestic and foreign laboratories.

Participated in and presented "Workshop on Animal Health Research of Common Interest to Mexico and USA" in Mexico City, where several international cooperative research projects on bovine respiratory disease were planned, April 2003.

Participated and presented in NCBA Animal Production Research Committee meeting at NADC October 2003.

Provided adenovirus diagnostic assistance (isolation of the virus, serotyping, serologic testing and antiserum to the prototype ovine and bovine adenoviruses) to scientists in domestic and foreign diagnostic laboratories.

Provided technical assistance on deer adenovirus hemorrhagic disease to several state Departments of Fish and Game.

Provided technical assistance on parainfluenza virus type 3 and bovine respiratory syncytial virus to scientists at a state university to study immune reaction to virus infection in the lung of small ruminants.

Served as reviewers for manuscripts submitted to refereed national and international scientific publications.

Corresponded with scientists (Veterinary Medical Research Institute, Hungarian Academy of Sciences) in Budapest, Hungary, on the Adenovirus Study Group of the International Committee on Taxonomy of Viruses on adenovirus taxonomy and classification. Results from our laboratory have helped to establish a new genus *Atadenovirus* in the *Adenoviridae* family.

Attended the joint meetings of the American Association of Veterinary Laboratory Diagnosticians and United States Animal Health Association and participated as a member of the Diagnostic Virology Committee (AAVLD) and the Committee on Infectious Diseases of Cattle, Bison and Lama (Vice Chair of Committee) and Committee on Sheep and Goats (USAHA).

Participate and organize the Meeting of the United States-Japan Cooperative Program in Natural Resources Panel of Animal and Avian Health Meeting held in Ames, IA and Tsukuba Japan alternate years to discuss research common to the National Animal Disease Center and the National Institute for Animal Health.

Participated in organizing and conducting the meeting on "Diseases at the Interface Between Domestic Livestock and Wildlife Species" held in Ames, IA, July 17-18, 2003.

Participated as a member of the Sheep Health Committee of the National Institute for Animal Agriculture.

PUBLICATIONS:

Ackermann, M.R., Brogden, K.A. Review. Response of the ruminant respiratory tract to Mannheimia (Pasteurella) haemolytica. Microbes and Infection. 2000. v. 2. p. 1079-1088.

Al-Ghamdi, G.M., Ames, T.R., Baker, J.C., Walker, R., Chase, C.C.L., Frank, G.H., Maheswaran, S.K. Serotyping of Mannheimia (Pasteurella) haemolytica isolates from the upper Midwest United States. Journal of Veterinary Diagnostic Investigations. 2000. v. 12. p. 576-578.

Belzer, C.A., Tabatabai, L.B., Frank, G.H. Purification and characterization of the Pasteurella haemolytica 35 kilodalton periplasmic iron-regulated protein. Preparative Biochemistry and Biotechnology. 2000. v. 30(4). p. 343-355.

Boyce, W.M., Woods, L.W., Keel, K., MacLachlan, N.J. Porter, C.O., Lehmkuhl, H.D. An epizootic of adenovirus induced hemorrhagic disease in captive black-tailed deer (Odocoileus hemionus). Journal of Zoo and Wildlife Medicine. 2000. v. 31(3). p. 370-373.

Brogden, K.A. Bacterial evasion of host-derived antimicrobial peptides on mucosal surfaces. Brogden, K.A., et al., editors. Virulence Mechanisms of Bacterial Pathogens, 3rd Edition. American Society for Microbiology Press. 2000. p. 19-40.

Debey, B.M., Lehmkuhl, H.D. Fatal infections in lambs associated with an adenovirus. 43rd Annual Meeting, American Association of Veterinary Laboratory Diagnosticians. 2000. Abstr. p. 16.

Frank, G.H., Briggs, R.E., Loan, R.W., Purdy, C.W., Zehr, E.S. Effects of tilmicosin treatment on Pasteurella haemolytica organisms in nasal secretion specimens of calves with respiratory tract disease. American Journal of Veterinary Research. 2000. v. 61. p. 525-529.

Frank, G.H., Duff, G.C. Effects of tilmicosin phosphate administered prior to transport or at time of arrival and feeding of chlortetracycline after arrival in a feedlot on Mannheimia haemolytica organisms in nasal secretions of transported steers. American Journal of Veterinary Research. 2000. v. 61. p. 1479-1483.

Fulton, Robert W., Purdy, C.W., Confer, A.W., Saliki, J.T., Loan, R.W., Briggs, R.E., and Burge, L.J. Bovine viral diarrhea viral infections in feeder calves with respiratory disease: Interactions with Pasteurella spp., parainfluenza-3 virus, and bovine respiratory syncytial virus. Canadian Journal of Veterinary Research. 2000. v. 64. p. 151-159.

Jeyaseelan, S., Kannan, M.S., Hsuan, S.L., Walseth, T.F., Singh, A.K., Briggs, R.E., Maheswaran, S.K. Role of arachidonic acid metabolites in Pasteurella haemolytica leukotoxin-induced cytolysis. Conference for Research Workers in Animal Disease. 2000. Abstr. #148.

Lapointe, J.M., Woods, L.W., Lehmkuhl, H.D., Keel, K., Rossitto, P.V., Swift, P.K., MacLachlan, N.J. Serologic detection of adenoviral hemorrhagic disease in black-tailed deer in California. Journal of Wildlife Diseases. 2000. v. 36(2). p. 374-377.

Lee, H.Y., Kehrli, Jr., M.E., Brogden, K.A., Gallup, J.M., Ackermann, M.R. Influence of beta 2-integrin adhesion molecule expression and pulmonary infection with Pasteurella haemolytica on cytokine gene expression in cattle. Infection and Immunity. 2000. v. 68(7). p. 4274-4281.

Leite, F., Brown, F.J., Sylte, M.J., Briggs, R.E. and Czuprynski, C.J. Recombinant bovine interleukin-1 beta amplifies the effects of partially purified Pasteurella haemolytica leukotoxin on bovine neutrophils in a beta-2-integrin-dependent manner. Infection and Immunity. 2000. v. 68. p. 5581-5586.

Purdy, C.W., Loan, R.W., Straus, D.C., Briggs, R.E., Frank, G.H. Conglutinin and immunoconglutinin titers in stressed calves in a feedlot. American Journal of Veterinary Research. 2000. v. 61(11). p. 1403-1409.

Ramirez-Romero, R., Brogden, K.A., Gallup, J.M., Dixon, R.A.F., Ackermann, M.R. Reduction of pulmonary mast cells in areas of acute inflammation in calves with Mannheimia (Pasteurella) haemolytica pneumonia. Journal of Comparative Pathology. 2000. v. 123. p. 29-35.

Ramirez-Romero, R. and Brogden, K.A. The potential role of the Arthus and Shwartzman reactions in the pathogenesis of pneumonic pasteurellosis. Inflammatory Research. 2000. v. 49. p. 98-101.

Saliki, J.T. Caseltine, S.L. Fulton, R.W., Lehmkuhl H.D. Isolation of bovine adenovirus type 7 from calves with respiratory disease. 43rd Annual Meeting, American Association of Veterinary Laboratory Diagnosticians. 2000. Abstr. p. 88.

Sorden, S.D., Woods, L.W., Lehmkuhl, H.D. Fatal pulmonary edema
in white-tailed deer (Odocoileus virginianus) associated with adenovirus infection. Journal of Veterinary Diagnostic Investigation. 2000. v. 12(4). p. 378-380.

Storz, J., Lin, M., Purdy, C.W., Chouljenko, V.N., Kousoulas, K.G., Enright, F.M., Gilmore, W.C., Briggs, R.E., Loan, R.W. Coronavirus and Pasteurella infections in bovine shipping fever pneumonia and Evans criteria for causation. Journal of Clinical Microbiology. 2000. v. 38.
p. 3291-3298.

Storz, J., Purdy, C.M., Xiaoqing, L., Burrell, M., Truax, R.E., Briggs, R.E., Frank, G.H., Loan, R.W. Isolation of respiratory bovine coronavirus, other cytocidal viruses, and Pasteurella spp from cattle involved in two natural outbreaks of shipping fever. Journal of the American Veterinary Medical Association. 2000. v. 216. p. 1599-1604.

Thumbikat, P. Jeyaseelan, S., Briggs, R.E., Kannan, M.S., Maheswaran, S.K. Functional dissection of Pasteurella haemolytica leukotoxin mutants. Conference for Research Workers in Animal Disease. 2000. Abstr. #147.

Williams, E.S., Lehmkuhl, H.D., Kreeger, T., Hearne, C., Cavender, J., Van Campen, H. Fatal systemic adenovirus infection in a captive moose calf (Alces alces). 49th Annu. Conference of Wildlife Diseases Association. 2000. Abstr. #21, p.38.

Ackermann, M.R., Mohammadi, G., Grubor, B., Gallup, J.M., Brogden, K.A., Barua A.B., and Olson, J.A. Lack of Toxicity in Lambs Receiving Repeated, High Doses of Retinoyl b-Glucuronide (Rag) Intravenously. Functions and Actions of Retinoids and Carotenoids: Building in the Vision of James Allen Olson. 2001. Iowa State University, Ames, IA. p. 35.

Brogden, K.A., Kalfa, V.C., Ackermann, M.R., Palmquist, D.E., McCray, Jr., P.B., Tack, B.F. The ovine cathelicidin SMAP29 kills ovine respiratory pathogens in vitro and in an ovine model of pulmonary infection. Antimicrobial Agents and Chemotherapy. 2001. v. 45. p. 331-334.

Brogden, K.A., Heidari, M., Sacco, R.E., Guthmiller, J.M., Johnson, G.K., Jia, H.P., McCray, Jr., P.B., Tack, B.F. Beta Defensin-induced Adaptive Immune Response to Ovalbumin in Mice. 101st American Society for Microbiology 2001. Abstract p. 344-345.

Caverly, J.M., Radi, Z.A., Andreasen, C.B., Doxon, R.A., Brogden, K.A., Ackermann, M.R. Comparison of bronchoalveolar lavage fluid obtained from Mannheimia haemolytica-inoculated calves with and without prior treatment with the selectin inhibitor TBC1269. American Journal of Veterinary Research. 2001. v. 62. p. 665-672.

DeBey, B.M., Lehmkuhl, H.D., Chard-Bergstrom, C., Hobbs, L.A. Ovine adenovirus serotype 7-associated mortality in lambs in the United States. Veterinary Pathology. 2001. v. 38(6). p. 644-648.

Frazier, M., Pence, M., Mauel, M.J., Ligget, A., Hines, M.E., Sangster, L., Lehmkuhl, H.D., Miller, D., Styer, E., West, J., Baldwin, C.A. Endometritis in postparturient cattle associated with bovine herpesvirus-4 infection: 15 cases. Journal of Diagnostic Investigation. 2001. v. 13(6). p. 502-508.

Heidari, M., Brogden, K.A. Anionic Antimicrobial Peptide May Originate as
a Larger Precursor Protein. 101st American Society for Microbiology. 2001. Abstract p. 333.

Kalfa, V.C., Jia, H.P., Kunkle, R.A., McCray, Jr., P.B., Tack, B.F., Brogden, K.A. Congeners of SMAP29 kill ovine pathogens and induce ultrastructural damage in bacterial cells. Antimicrobial Agents of Chemotherapy. 2001. v. 45. p. 3256-3261.

Kalfa, V.C., Palmquist, D., Ackermann, M.R., Brogden, K.A. Suppression of Mannheimia (Pasteurella) haemolytica serovar 1 infection in lambs by intrapulmonary administration of ovine antimicrobial anionic peptide. International Journal of Antimicrobial Agents. 2001. V. 17. p. 505-10.

Lehmkuhl, H.D., DeBey, B.M., Cutlip, R.C. A new serotype adenovirus isolated from a goat in the United States. Journal of Veterinary Diagnostic Investigation. 2001. v. 13(3). p. 195-200.

Lehmkuhl, H.D., Woods, L.W. Characterization of a new adenovirus species isolated from black-tailed deer in California. Archives of Virology. 2001. v. 146(6). p. 1187-1196.

Radi, Z.A., Caverly, J.M., Dixon, R.A., Brogden, K.A., Ackermann, M.R. Effects of the synthetic selectin inhibitor TBC1269 on tissue damage during acute Mannheimia haemolytica-induced pneumonia in neonatal calves. American Journal of Veterinary Research. 2001. v. 62. p. 17-22.

Ramirez-Romero, R., Brogden, K.A., Gallup, J.M., Sonea, I.M., Ackermann, M.R. Mast cell density and substance P-like immunoreactivity during the initiation and progression of lung lesions in ovine Mannheimia (Pasteurella) haemolytica pneumonia. Microbial Pathogenesis. 2001. v. 30. p. 325-335.

Rimler, R.B., Brogden, K.A. Ultrastructural location of the cross-protection factor on in vivo and in vitro grown Pasteurella multocida. Avian Diseases. 2001. v. 45. p. 946-952.

Woods, L.W., Swift, P.K., Lehmkuhl, H.D., Chiu, P.H., Hanley, R.S., Nordhausen, R.W., Stillian, M.H., Drew, M.L. Experimental adenovirus hemorrhagic disease in white-tailed deer fawns. Journal of Wildlife Diseases. 2001. v. 37(1). p. 153-158.

Zehr, E.S., Frank, G.H., Tabatabai, L.B. Characterization of Proteins Extracted from the Outer Membrane of Mannheimia haemolytica by sepharose-bound bovine holotransferrin. 101st General Meeting of the American Society for Microbiology. 2001. Abstr. #100.

Zhao, C., Nguyen, T., Lui, L., Sacco, R.E., Brogden, K.A., Lehrer, R.I. Gallinacin-3, an inducible epithelial beta-defensin in the chicken. Infection and Immunity. 2001. v. 69. p. 2684-2691.

Brogden, K.A. Polymicrobial diseases of animals and humans. Brogden, K.A., Guthmiller J.M., editors. ASM Press, Washington, DC. Polymicrobial Diseases. 2002. p. 3-20.

Brogden, K.A., Guthmiller, J.M. Polymicrobial diseases: current and future research. Polymicrobial Diseases. Brogden, K.A., Guthmiller J.M., editors. ASM Press, Washington, DC. 2002. p. 403-410.

Drake, D.R., Brogden, K.A. Continuous culture chemostat systems and flowcells as methods to investigate microbial interactions. Brogden, K.A., Guthmiller J.M., editors. ASM Press, Washington, DC. Polymicrobial Diseases. 2002. p. 21-30.

Fales-Williams, A.J., Gallup, J.M., Ramirez-Romero, R., Brogden, K.A., Ackermann, M.R. Increased anionic peptide distribution and intensity during progression and resolution of bacterial pneumonia. Clinical Diagnostic Laboratory Immunology. 2002. v. 1. p. 28-32.

Fales-Williams, A.J., Brogden, K.A., Huffman, E., Gallup, J.M., Ackermann, M.R. Cellular distribution of anionic antimicrobial peptide in normal lung and during acute pulmonary inflammation. Veterinary Pathology. 2002. v. 39(6). p. 706-711.

Fent, G.M., Fulton, R.W., Saliki, J.T., Caseltine, S.L., Lehmkuhl, H.D., Confer, A.W., Purdy, D.W., Briggs, R.E., Loan, R.W., Duff, G.C. Bovine Adenovirus serotype 7 infections in postweaning calves. American Journal of Veterinary Research. 2002. v. 63(7). 976-978.

Frank, G.H., Briggs, R.E, Duff, G.C., Loan, R.W., Purdy, C.W. Effects of pretransit vaccination and arrival medication with florfenicol on the health of transported calves and the presence of Mannheimia haemolytica organisms in nasal secretions. American Journal of Veterinary Research. 2002. v. 63. p. 251-256.

Fulton, R.W., Ridpath, J.F., Saliki, J.T., Briggs, R.E., Confer, A.W., Burge, L.J., Purdy, C.W., Loan, R.W., Duff, G.C., Payton, M.E. Bovine viral diarrhea virus (BVDV) 1B: predominant BVDV subtype in calves with respiratory disease. Canadian Journal of Veterinary Research. 2002. 66(3). p. 181-190.

Grubor, B., Ramirez-Romero, R., Barua, A.B., Gallup, J.M., Crouch, E., Brogden, K.A., Ackermann, M.R. Surfactant protein D expression in normal and pneumonic ovine lung. 102nd American Society for Microbiology. 2002. p. 189. Abstract #E-12.

Heidari, M., Hamir, A.N., Cutlip, R.C., Brogden, K.A. Antimicrobial nionic peptide binds in vivo to *Mannheimia* (*Pasteurella*) *haemolytica* attached to ovine alveolar epithelium. International Journal of Antimicrobial Agents. 2002. v. 20. p. 69-72.

Lehmkuhl, H.D., Hobbs, L.A. Phylogenetic and serologic analysis of Atadenovirus. Proceedings of United States-Japan Cooperative Program in Natural Resources - Panel on Animal Health and Avian Health. 2002. p. 8-9.

Lehmkuhl, H.D. Bovine adenovirus. Section in: Animal Health and Production Compendium, 2002 Edition. Internet publication by The Commonwealth Agricultural Bureaux (CAB) International, Wallingford, UK.

Parker, J., Woods, L.W., Lehmkuhl, H.D., Stillian, M.H. Preliminary Investigation of squirrels as a possible reservoir for deer adenovirus. Wildlife Disease Conference. 2002. p. 98. Abstract #74.

Radi, Z.A., Brogden, K.A., Dixon, R.A., Gallup, J.M., Ackermann, M.R. A selectin inhibitor decreases neutrophil infiltration during acute *Mannheimia haemolytica* pneumonia. Veterinary Pathology. 2002. v. 39(6). p. 697-705.

Shilton, C.M., Smith, D.A., Woods, L.W., Crawshaw, G.J., Lehmkuhl, H.D. Adenoviral infection in captive moose (*Alces Alces*) in Canada. Journal of Zoo and Wildlife Medicine. 2002. v. 33(1). p. 73-79.

Swift, P.K., Woods, L.W., Lehmkuhl, H.D., Jones, K.R. An update of adenoviral hemorrhagic disease in Mule Deer in California. Wildlife Disease Conference. 2002. p. 144. Abstract #115.

Ward, A.C.S., Weiser, G.C., Delong, W.J., Frank, G.H. Characterization of Pasteurella spp isolated from healthy domestic pack goats and evaluation of the effects of a commercial Pasteurella vaccine. American Journal of Veterinary Research. 2002. v. 63. p. 119-123.

Zakhartchouk, A., Bout, A., Woods, L.W., Lehmkuhl, H.D., Havenga, J.E. Odocoileus hemionus deer adenovirus is related to the members of Atadenovirus genus. Archives of Virology. 2002. v. 147(4). p. 841-847.

Brodgen, K.A., Heidairi, M., Sacco, R.E., Palmquist, D.E., Guthmiller, J.M., Johnson, G.K., Jia, H.P., Tack, B.F., McCray, JR., P.B. Defensin-induced adaptive immunity in mice and its potential in preventing periodontal disease. Oral Microbiology and Immunology. 2003. v. 18. p. 95-99.

Brogden, K.A., Guthmiller, J.M. Polymicrobial diseases, a concept whose time has come. ASM News. 2003. v. 69(2). p. 69-73.

Calvery, J.M., Diamond, G., Gallup, J.M., Brogden, K.A., Dixon, R.A., Ackermann, M.R. Coordinated expression of tracheal antimicrobial peptide and inflammatory-response elements in the lungs of neonatal calves with acute bacterial pneumonia. Infection and Immunity. 2003. Infection and Immunity. v. 71(5). p. 2950-2955.

Fajt, V.R., Apley, M.D., Roth, J.A., Frank, D.E., Brogden, K.A., Skogerboe,T.L., Shostrom, V.K., Chin, Y.L. The effects of danofloxacin and tilmicosin on neutrophil function and lung consolidation in beef heifer calves with induced *Pasteurella* (*Mannheimia*) *haemolytica* pneumonia. Journal of Veterinary Pharmacology and Therapeutics. 2003. v. 26(3). p. 173-179.

Frank, G.H., Briggs, R.E., Duff, G.C., Hurd, H.S. Effects of intranasal exposure to leukotoxin-deficient *Mannheimia haemolytica* at the time of arrival at the feedyard on the subsequent isolation of *M. haemolytica* from nasal secretions of calves. American Journal of Veterinary Research. 2003. v. 64(5). p. 580-585.

Fulton, R.W., Step, D.L., Ridpath, J.F., Saliki, J.T., Confer, A.W., Johnson, B.J., Briggs, R.E., Hawley, R.V., Burge, L.J., Payton, M.E. Response of calves persistently infected with noncytopathic bovine viral diarrhea virus (BVDV) subtype 1B after vaccination with heterologous BVDV strains in modified live virus vaccines and *Mannheimia haemolytica* bacterin-toxoid. Vaccine. 2003. v. 21. p. 2980-2985.

Gallup, J.M., Grubor, B., Barua, A.B., Mohammadi, G., Brodgen, K.A., Olson, J.A., Ackermann, M.R. Repeated intravenous doses of all trans-retinoyl beta-D-glucuronide is not effective in the treatment of bacterial bronchopneumonia in lambs but is devoid of gross and acute toxicity. Medical Science Monitor. 2003. v. 8(9). p. 345-353.

Fulton, R.W., Briggs, R.E., Payton, M.E., Confer, A.W., Saliki, J.T., Ridpath, J.F., Burge, L.J., Duff, GC. Maternally derived humoral immunity to bovine viral diarrhea virus (BVDV)1a, BVDV1b, BVDV2, bovine herpesvirus-1, parainfluenza-3 virus bovine respiratory syncytial virus, *Mannheimia haemolytica* and *Pasteurella multocida* in beef calves, antibody decline by half-life studies and effect on response to vaccination. Vaccine. Jan 26;22(5-6): 643-9, 2004.

Respiratory Diseases of Livestock

Project Title: Construction of Live Mucosal and Injectable Bacterial Vaccines Against Avian and Bovine Respiratory Diseases
CRIS Number:: 3625-32000-067-03T
Scientists: Tatum, F., Briggs, R.
Location: Ames, Iowa
National Animal Disease Center
Respiratory Diseases of Livestock
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PROJECT OBJECTIVES:
Pasteurella spp. are part of the normal nasal flora in cattle and are also present in lower numbers in the trachea. As a consequence of shipping and feedlot stresses, Mannheimia haemolytica (Pasteurella haemolytica) 1 increase on upper respiratory tract mucosa and upon establishment in lungs result in pneumonia. M. haemolytica produces several virulence factors, foremost of which is leukotoxin, a member of the related family of toxins designated as RTX (repeated toxins). Other bacteria associated with bovine respiratory disease include Pasteurella multocida, Haemophilus somnus, and Mycoplasma spp.

We have developed the tools (temperature-sensitive plasmids) and acquired the requisite genetic understanding (characterized restriction-modification systems) to genetically engineer members of the Pasteurellaceae, a diverse group of bacteria causing a wide range of diseases in animals and man. The replication-conditional plasmids, pBB192 and pBBD80, have allowed us to construct 'clean' deletion mutant vaccine candidates of M. haemolytica, P. multocida, and Haemophilus somnus. Such mutants are devoid of exogenous DNA and considered by NIH guidelines to be non-recombinant organisms.

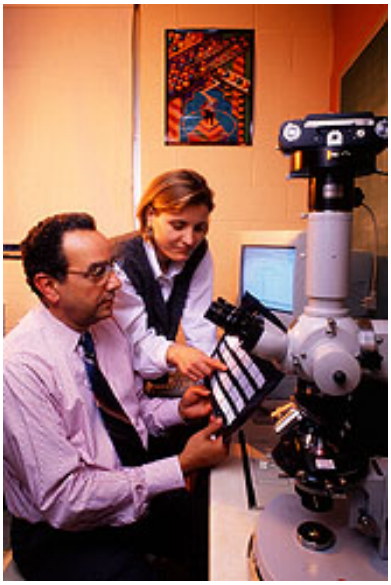
OVERALL PROJECT ACCOMPLISHMENTS:
A new mucosal vaccine was developed and tested, in cooperation with New Mexico State University, which protected cattle from pneumonic Pasteurellosis within 3 days of administration. The vaccine was effective either by top-dressing on feed or by intranasal administration at the point of first assembly. The vaccine offers hope to many cattle producers who need a fast and effective vaccine and to the cattle industry which needs to avoid adverse reactions to injected vaccines.

IMPACT:

TECHNOLOGY TRANSFERS:

PUBLICATIONS:

Avian Disease and Oncology Laboratory (ADOL)
East Lansing, Michigan



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PROJECTS

Avian Disease and Oncology Laboratory (ADOL)

- Genomics and Immunogenetics of Economically Important Traits of Poultry
- Strategies for Reduction of Tumors and Viral Shedding in Marek's Disease
- Epidemiology, Diagnosis and Control of Retrovirus Infection in Poultry

Avian Disease and Oncology Laboratory

Project Title: Genomics and Immunogenetics of Economically Important Traits of Poultry
CRIS Number: 3635-31320-007-00D
Scientists: Cheng, H., Zhang, H., Hunt, H.
Location: East Lansing, MI, Avian Disease and Oncology Research
Contact: Cheng, H., Phone: (517) 337-6758, Fax: (517) 337-6776, hcheng@msu.edu

PROJECT OBJECTIVES:

Poultry is the third largest agricultural commodity and at 41%, the main meat consumed in the U.S. The utilization of advanced breeding methods and large-scale industrialization of animal production have enabled tremendous progress in economic efficiency. The development of large-scale hatcheries and poultry farms has prompted increased efforts in disease control. Combinations of disease agent eradication, chemoprophylaxis, vaccination, and sanitation have helped to control but not eliminate losses due to disease. Virus-induced tumor diseases of particular concern and importance to the poultry industry are being studied at this location. Marek's disease (MD) is a lymphoproliferative disease (T cell cancer) caused by a member of the herpesvirus family. B cell cancers are found in avian leukosis virus (ALV)-infected chickens. These diseases cost poultry producers millions of dollars each year due to condemnation of the birds and reduced egg production. Control of MD has been achieved through the use of vaccines that prevent tumor development but do not block MDV infection. Control of ALV is conducted by eliminating virus-positive individuals to eradicate future infections. With regard to MD, the constant presence of the pathogen, periodic outbreaks in vaccinated flocks, the emergence of new and highly virulent strains, and the lack of new vaccines all point to the need for continued research on alternative methods for disease control. One attractive solution is breeding for resistance and/or improved vaccinal response. Although genetic resistance to MD is complex and controlled by many genes, genetic selection for high levels of resistance can be obtained. Our goal is to understand the molecular, biochemical and immunological basis of genetic resistance and vaccinal response to MD. Our multidisciplinary approach is to develop tools and reagents that can identify all the genes, proteins and pathways involved. Once obtained, this information will be useful to select chickens with superior disease resistance and response to vaccines, thereby, increasing animal health, making producers more competitive, and reducing the costs to consumers. In the case of ALV, besides eliminating virus-positive individuals to stop virus spread in infected flocks, two other measures should be considered to prevent future outbreaks: 1) use marker-assisted selection to increase the frequency of resistant birds, and. 2) periodic surveillance to monitor for new disease causing variants. Our aims are to define genes and alleles that confer viral resistance, understand the mechanisms for generating new ALV disease-causing variants, and develop chicken strains or selection schemes that would minimize the potential for producing new highly-virulent ALV strains.

OVERALL PROJECT ACCOMPLISHMENTS:
Since the inception of this project, the resources to identify agricultural important genes and the results from the application of these tools have grown rapidly. Using a White Leghorn by Jungle Fowl cross, over 1,200 genetic markers or genes have been placed that cover the entire chicken genome. This genetic map is the most comprehensive and widely used in the world. The map has been integrated with the other two chicken genetic maps to make a consensus map containing close to 2,000 markers as well as physical maps. The integrated genetic and physical maps formed the framework of the whole genome sequence that was released on March 2004. In addition, the genetic map was used to identify many regions containing genes that confer resistance to MD in both experimental and commercial animals. Unique lines have been developed at our location, which will greatly aid in the identification and biological characterization of the actual genes. New methods that look at gene expression variation and viral-chicken protein-protein interactions have been implemented that will complement our existing efforts. Using a screen to identify virus-chicken protein interactions, it was determined that chicken growth hormone and stem cell antigen 2 interacts with viral proteins to modulate the immune response, and both confer disease resistance. These are the first MD resistance genes identified. With respect to immunology, the molecular characterization of MHC genes in eight B congenic strain was completed. A new method was developed to make reagents that identify MHC markers on cells. These reagents helped to demonstrate that MHC gene expression is reduced in MD virus-infected cells. An antiserum known as R2 was developed that detects endogenous virus (EV) expression, which is valuable since EV expression has been correlated with decreased egg and meat production, susceptibility to viral-induced tumors, and possibly the generation of ALV variants. Furthermore, a genetic assay was developed that identifies chickens resistant to ALV, which enables assessing genetic risk of susceptibility to subgroup B, D, and E ALV in chicken flocks without involving actual viral challenge. More importantly, it drastically simplifies and accelerates selection for genetically resistant (or susceptible) lines of chickens to the ALV subgroups. Finally, a new method has been developed to culture early embryonic cells, which following genetic manipulations, are still able to properly populate the sex organs of chimeric chickens.

IMPACT:
Our genetic map provided the framework for the chicken genome sequence. With our genetic map, investigators have been able to identify genomic regions containing genes of agricultural importance. Our efforts have also demonstrated how combining functional genomic screens can identify the causative genes, which allows for more accurate breeding using molecular markers and the elucidation of biological pathways. Our unique reagents and chicken lines also empower scientists, especially with respect to immunological characterization.

TECHNOLOGY TRANSFER:
The majority of genetic markers were developed by our location and are being used throughout the world. Markers for specific Marek's disease resistance genes are being evaluated in commercial layers.

PUBLICATIONS:

Bacon, L.D., Hunt, H.D. and Cheng, H.H. A review of the development of chicken lines to resolve genes determining resistance to diseases. Poultry Science. 2000. v. 79. p. 1082-1093.

Bacon, L.D. Detection of endogenous avian leukosis virus envelope in chicken plasma using R2 antiserum. Avian Pathology. 2000. v. 29. p. 153-164.

Bacon, L.D. Production of neutralizing antibody to ALV-J differs in hens from seven B-congenic white leghorn lines. Proceedings International Symposium on ALV-J and Other Retroviruses. 2000. p. 115-126.

Schmid, M., Nanda, I, Guttenbach, M., Steinlein, C., Hoehn, H., Schartl, M., Haaf, T., Weigend, S., Fries, R., Buerstedde, J.M., Wimmers, K., Burt, D.W., Smith, J., A'Hara, S., Law, A., Griffin, D.K., Bumstead, N., Kaufman, J., Phomsom, P.A., Burke, T., Groenen, M.A.M., Crooijmans, R.P.M.A., Vignal, A., Fillon, V., Morisson, M., Pitel, F., Tixier-Boichard, M., Ladjali-Mohammedi, K., Hillel, J., Maki-Tanila, A., Cheng, H.H., Delany, M.E., Burnside, J., and Mizuno, S. First report on chicken genes and chromosomes. Cytogenetics and Cell Genetics. 2000. v. 90. p. 169-218.

Fulton, J.E., Hunt, H.D., and. Bacon, L.D. Chicken major histocompatibility complex class I definition using antisera induced by cloned class I sequences. Poult. Sci. 2001. v.80. p.1554-1561..

Bacon, L.D., H. D. Hunt, and H. H. Cheng. Genetic resistance to Marek's disease. In: Current Topics in Microbiology and Immunology. K. Hirai, ed. Springer-Verlag, Berlin. pp. 121-142. 2001.

Liu, H.-C., Kung, H.J., Fulton, J.E., Morgan, R.M., Cheng, H.H. Growth hormone interacts with the Marek's disease virus SORF2 protein and is associated with disease resistance in chicken. Proceedings of the National Academy of Sciences USA. 2001. v. 98. p. 9203-9208.

Suchyta, S.P., Cheng, H.H., Burnside, J., and Dodgson, J.B. Comparative mapping of chicken anchor loci orthologous to genes on human chromosomes 1, 4 and 9. Animal Genetics. 2001. v. 32. p. 12-18.

Yonash, N., Cheng, H.H., Hillel, J., Heller, E.D., and Cahaner, A. DNA microsatellites linked to quantitative trait loci affecting antibody response and survival rate in meat-type chickens. Poultry Science. 2001. v. 80. p. 22-28.

Zhu, J., Lillehoj, H.S., Cheng, H.H., Pollock, D., and Emara, M.G. Screening for highly heterozygous chickens in outbred commercial broiler lines to increase detection power for mapping quantitative trait loci. Poultry Science. 2001. v. 80. p. 6-12.

Liu, H.C., Cheng, H.H., Sofer, L., Burnside, J. Identification of genes and pathways for resistance to Marek's disease through DNA microarrays. Proceedings of 6th International Symposium on Marek's disease. 2001. p. 157-162.

Ambady, S., Cheng, H.H., Ponce de Leon, F.A. Development and Mapping of Microsatellite Markers Derived from Chicken Chromosome-Specific Libaries. Poultry Science. 2002. v. 81. p. 1644-1646.

Lipkin, E., Fulton, J., Cheng, H.H., Yonash, N., Soller, M. Quantitative Trait Locus Mapping in Chickens by Selective DNA Pooling and Dinucleotide Microsatellite Markers by Using Purified DNA and Fresh or Frozen Red Blood Cells as Applied to Marker-Assisted Selection. Poultry Science. 2002. v. 81. p. 283-292.

Yonash, N., Bacon, L.D., Smith, E. Concentration of Immunoglobulin G in Plasma Varies Among 6C.7 Recombinant Congenic Strains in Chickens. Poultry Science. 2002. v. 81. p. 1104-1108.

Bacon, L.D., Zajchowski, L., Clark, M.E., Etches, R.J. Identification and Evaluation of Major Histocompatibility Complex Antigens in Chicken Chimeras and Their Relationship to Germline Transmission. Poultry Science. 2002. v. 81. p. 1427-1438.

Bacon, L.D., Palmquist, D.E. Chicken Lines Differ in Production of Interferon-Like Activity by Peripheral White Blood Cells Stimulated with Phytohemagglutinin. Poultry Science. 2002. v. 81. p. 1629-1636.

Cheng, H.H. Selection for Disease Resistance: Molecular Genetic Techniques. Poultry Breeding and Technology. 2003. p. 385-398.

Cheng, H.H. Molecular genetic techniques for identifying disease resistance genes. Muir, W.M., Aggrey, S.E., editors. CABI Publishing, Cambridge, MA. Poultry Breeding and Biotechnology. 2003. p. 385-398.

Liu, H.C., Niikura, M., Fulton, J., Cheng, H.H. Identification of chicken stem lymphocyte antigen 6 complex, locus E (*LY6E*, alias *SCA2*) as a putative Marek's disease resistance gene via a virus-host protein interaction screen. Cytogenetics and Genome Research. 2003. v. 102. p. 304-308.

Liu, H., Cheng, H.H. Genetic Mapping of the Chicken Stem Cell Antigen 2 (SCA2) Gene to Chromosome 2 Via PCR Primer Mutagenesis. Animal Genetics. 2003. v. 34. p. 158-160.

Zhu, J.J., Lillehoj, H.S., Allen, P.C., Van Tassell, C.P., Sonstegard, T.S., Cheng, H.H., Dollock, D., Sadjadim, M., Min, W., Emara, M.G. Detection of a QTL Associated with Eimeria-Oocyst Shedding in Commercial Chickens. Poultry Science. 2003. v. 8. p. 9-16.

Zhang, H.M., Cheng, H.H. SNP identification and genetic mapping of chicken *ephrin type-B receptor 2* gene to linkage group E54. Anim. Genet. 2004. v. 35. p. 162-163.

Zhang, H.M., Cheng, H.H. Chicken *Tubby-like protein 1* (*TULP1*) gene maps to chromosome 26. 2004. Anim. Genet. v. 35. p. 165-166.

Bacon, L.D., Fulton, J.E., and Kulkarni, G.B. Methods for evaluating and developing commercial chicken strains free of endogenous, subgroup E avian leukosis virus. Avian Pathology. 2004. v. 32. 2333-243.

Avian Disease and Oncology Laboratory

Project Title: Strategies for Reduction of Tumors and Viral Shedding in Marek's Disease
CRIS Number: 3635-32000-012-00D
Scientists: Silva, R., Lee, L., Hunt, H., Vacant, Vacant
Location: East Lansing, MI, Avian Disease and Oncology Research
Contact: Silva, R., Phone: (517) 337-6833, Fax: (517) 337-6776, silvar@msu.edu

PROJECT OBJECTIVES:
Marek's disease (MD) is a lymphoproliferative and immunodepressive disease of chickens caused by a herpesvirus. Infection with the causative virus is nearly universal among commercial chicken flocks worldwide. The virus is relatively stable in the environment, is spread through the air and is unlikely to be eradicated from commercial flocks. Despite widespread use of vaccines, economic losses from mortality of layers and breeders and condemnation of broilers continue to plague producers. Losses tend to be sporadic and unpredictable. Moreover, the virus continues to mutate to greater virulence, reducing the effectiveness of many existing vaccines and causing concerns that vaccines currently considered effective will not protect in the future. Also, the host range of the virus has apparently expanded to include adult chickens and turkeys. The major problem is the need for practical and cost-efficient technology that will better control losses, both now and in the future. This research project addresses the problem through a comprehensive program dealing with basic mechanisms of the disease process, characterization of the virus, and development of better vaccines. Much of this work is also designed to complement and improve the genetic resistance of chickens to this disease.

OVERALL PROJECT ACCOMPLISHMENTS:
We confirmed that recent MDV isolates are more virulent than earlier isolates and that there are some changes in the MDV genome that may be indicative of different pathotypes. We also compared some highly attenuated, moderately attenuated and some low virulent strains for their ability to protect against an MDV challenge. We found that partly attenuated, slightly virulent strains provided the best protection. These findings will help us to better design new vaccines. We also demonstrated that the putative oncogene of MDV (meq), could be deleted and that meq is essential for transformation of lymphocytes in infected chickens. Using mutational analysis, we demonstrated that the pp38 gene of the vaccine virus, Rispens, functions identically to the pp38 gene in the very virulent Md5 virus. This knowledge helps us to understand the mechanisms by which the virus replicates, causes disease, and may be useful in developing a new class of recombinant DNA vaccines.

IMPACT:
Research conducted on various aspects of Mareks' disease will undoubtedly lead to development of better methods for its control. Successful control of Marek's will insure the viability of the United States poultry industry and insure a supply of inexpensive meat and egg products of high quality for consumers in the U.S. and in other countries.

TECHNOLOGY TRANSFER:
We developed a set of PCR primers that will specifically detect MDV in infected birds. These primers and the technology to use the primers have been sent to numerous state diagnostic labs, private poultry companies, and scientists at academic institutes. We have shared specific MD viral sequences with other scientists to enhance

the global effort to elucidate the function of various viral genes. We have also supplied characterized MD viral field strains to poultry companies for use in challenge experiments to test the susceptibility of different chicken strains and the efficacy of different vaccines. An MDV with a pp38 deletion was sent to a scientist to study the role of pp38 on cytotoxic lymphocytes. We sent the MDV cosmid clones to other laboratories to transfer the technology of MDV mutants to the scientific community.

PUBLICATIONS:

Lee, L.F., Wu, P., Sui, D., Ren, D., Kamil, J., Kung, H.J., and Witter R.L. The complete unique long sequence and the overall genomic organization of the GA strain of Marek's disease virus. Proc. Natl. Acad. Sci. USA. 2000. v. 97. p. 6091-6096.

Reddy, S.M., Witter, R.L., and Gimeno, I.M. Development of a quantitative-competitive polymerase chain reaction assay for serotype 1 Marek's disease virus. Avian Diseases. 2000. v. 44. p. 770-775.

Witter, R.L. and Morgan, R.W.. Marek's disease: recent developments and current status. Proc. 50th Western Poultry Disease Conference. 2001. p. 22-27.

Witter, R.L. Part II: Marek's disease vaccines - how the virus fights back. World Poultry . 2001. v. 17. p. 38-39

Witter, R.L. Protective efficacy of Marek's disease vaccines. In: Current Topics in Microbiology and Immunology. K. Hirai, ed. Springer-Verlag, Berlin. 2001. p. 58-90.

Witter, R.L. Recent advances in Marek's disease research. Proceedings. XII World Veterinary Poultry Congress. 2001. p. 9-15.

Witter, R.L. Part 1: Marek's disease vaccines - the viral battlefield. World Poultry. 2001. v. 17. p. 42-44.

Witter, R.L. Marek's disease vaccines - past, present and future (Chicken vs virus - a battle of the centuries). In: Proceedings of the 6th International Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amererican Association of Avian Pathologists, Kennett Square, PA. 2001. p. 1-9.

Silva, R.F., Lee, L. F. and Kutish, G. F. The genomic structure of Marek's disease virus. In: Current Topics in Microbiology and Immunology. K. Hirai, ed. Springer-Verlag, Berlin. 2001. p. 143-158.

Wu, P., Reed, W.M., Lee, L.F. Glycoproteins H and L of Marek's disease virus form a hetero-oligomer essential for translocation and cell surface expression. Archives of Virology. 2001. v. 146. p. 983-992.

Reddy, S.M., Lupiani, B., Silva,R. F., Lee, L. F., and R. L. Witter. Genetic manipulation of a very virulent strain of Marek's disease virus. In: Proceedings of the 6th International Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. American Association of Avian Pathologists, Kennett Square, PA. 2001. p. 55-57.

Wu, P, Reed, W.M., Lee, L.F., A study on the interaction of gH and gI of MDV. Proceedings of 6th

International Symposium on Marek's disease. 2001. p. 99-102.

Reddy, S.M., Sui, D., Wu P., Qin , A., and Lee.L. Sequence analysis of Marek's disease virus gene encoding the major capsid protein. In: Proceedings of the 6th International Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amererican Association of Avian Pathologists, Kennett Square, PA. 2001. p. 215-218.

Silva, R.F., Lee, L. F., and Kutish, G. F.. From gene to protein: what is new in Marek's disease. In: Proceedings of the 6th International Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. American Association of Avian Pathologists, Kennett Square, PA. 2001. p. 77-80.

Lupiani, B., Silva, R. F., Lee, L. F., and Reddy, S. M. Characterization of the Uss/TRrs junction region of different MDV pathotypes. In: Proceedings of the 6th International Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. American Association of Avian Pathologists, Kennett Square, 2001.PA. p. 51-53.

Lupiani, B., Lee, L. F., and Reddy, S. M.. Protein-coding content of the sequence of Marek's disease virus. In: Current Topics in Microbiology and Immunology. K. Hirai, ed. Springer-Verlag, Berlin. 2001. p. 159-190.

Miles, A.M., S. Reddy, M., and Morgan, R.W. Coinfection of specific-pathogen-free chickens with Marek's disease virus (MDV) and chicken infectious anemia virus: effect of MDV pathotype. 2001. Avian Diseases. V. 45. p.:9-18..

Kung, H.J., Xia, L., Brunovskis, P., Li, D., Liu, J.L., Lee, L.F. Meq: An MDV-specific bZIP transactivator with transforming properties. Hirai K, editor. Springer-Verlag, Berlin. Current Topics in Microbiology and Immunology. 2001. p. 245-260.

Parcells, M.S., Lin, S.F., Dienglewicz, R.L., Majerciak, V., Robinson, D.R., Chen, H.C., Wu, Z., Dubyak, G.R., Brunovskis, P., Hunt, H.D., Lee, L.F., and Kung, H.J. Marek's disease virus (MDV) encodes an interleukin-8 homolog (vIL-8): Characterization of the vIL-8 protein and a vIL-8 deletion mutant MDV. J. Virol. 2001. v. 75. p, 5159-5173.

Hunt, H.D., Lupiani, B, Miller, M.M., Gimeno, I.M., Lee, L.F., and Parcells, M.S.. Marek's disease virus down regulates surface expression of MHV (B complex) class I (BF) glycoproteins during active but not latent infection of chicken cells. Virology. 2001. v. 282. p. 198-205.

Lee, L.F., Wu, P., Sui, D., Ren, D., Kamil J., Kung, H. J., and Witter, R. L.. Comparison of the genomic organization of the GA strain of Marek's disease virus with herpes simplex virus. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. 2001. p. 81-84.

Lee, L.F., Nazerian K., Witter, R.L., Wu, P., Yanagida, N., and Yoshida, S.. Marek's disease virus vaccines for protection against Marek's disease. 2001.. U. S. Patent Off. #6,322,780.

Kung, H-J., Lee, L.F., Liu, J-L., Lin, S-F. A comparative study of bZIP proteins in oncogenic herpesviruses:

Meq and k-bZIP. Proceedings of 6th International Symposium on Marek's disease. 2001. p. 141-148.

Kamil, J., Robinson, D., Lee, L.F., Kung, H-J. Marek's disease virus encodes a secreted lipase. Proceedings of 6th International Symposium on Marek's disease. 2001. p. 209-214.

Cui, Z., Qin, A., Cui, X., Du, Y., Lee, L.F. Molecular identification of 3 epitopes on 38kd phosporylated proteins of Marek's disease viruses. Proceedings of 6th International Symposium on Marek's disease. 2001. p. 103-108.

Gimeno, I.M., Witter, R.L., Hunt, H.D., Lee, L.F., Reddy S.M., and Neumann, U. Marek's disease virus infection in the brain: virus replication, cellular infiltration and major histocompatibility complex antigen expression. Vet. Pathology. 2001. V. 38. p.491-503.

Gimeno, I.M., Witter, R. L., Hunt, H. D., Lee, L. F., Reddy. S. M., and Neumann, U. Chronological study of brain alterations induced by a very virulent plus (vv+) strain of Marek's disease virus (MDV). In: Proceedings of the 6th International Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. American Association of Avian Pathologists, Kennett Square, PA. . 2001. p. 21-26.

Gimeno, I.M., R.L. Witter, H.D. Hunt, S.M. Reddy, and U. Neumann. Differential attenuation of the induction by Marek's disease virus of transient paralysis and persistent neurological disease: a model for pathogenesis studies. Avian Pathol. 30:397-409. 2001.

Borenshtein, R., Davidson, I., Kung, H. J., and . Witter, R. L. Evidence for in vivo integration of the avian leukosis virus, subgroup J long terminal repeat into the Marek's disease virus in experimentally dually-infected chickens. In: Proceedings of the 6th International Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amererican Association of Avian Pathologists, Kennett Square, PA. 2001. p. 193-200.

Buscaglia, C., Calnek, B. W., Witter, R. L, Risso, M. A., Villat, M. C., and Prio, M. V. Immunosuppressive potential of two Argentine pathotypes of Marek's disease virus isolates. In: Proc. 6th Intl. Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amer. Assn. Avian Pathologists, New Bolton Center, PA. 2001. p. 69-72.

Cardona, C.J., Nazerian, K., Reed, W.M., and Silva, R.F.. Characterization of a recombinant fowlpox virus expressing the native hexon of hemorrhagic enteritis virus. Virus Genes. 2001. v. 22. p. 353-361.

Cui, X., Wei, P., Lee, L. F., Holland, M., Silva, R. F. and . Kung, H. J. Characterization of the expression of the Marek's disease virus meq gene in a recombinant baculovirus. In: Proceedings of the 6th International Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. Amererican Association of Avian Pathologists, Kennett Square, PA. pp. 135-139. 2001.

Dudnikov, L.A. and Witter, R. L.. A comparison of autologous and heterologous vaccination against Marek's disease. In: Proceedings of the 6th International Symposium on Marek's Disease. K.A. Schat, R.W. Morgan, M.S. Parcells and J.L. Spencer, eds. American Association of Avian Pathologists, Kennett Square, PA. 2001. p. 249-255.

Bacon, L.D., Witter, R.L., and Silva., R.F. Characterization and experimental reproduction of peripheral neuropathy in White Leghorn chickens. Avian Pathology. 2001. v.30 p.487-499..

Davidson, I., Borenshtain, R., Kung, H.J., and Witter, R.L. Molecular indications for *in vivo* integration of the avian leukosis virus, subgroup J-long terminal repeat into the Marek's disease virus in experimentally dually-infected chickens. Virus Genes. 2002. v. 24. p. 173-180.

Witter, R.L. Induction of strong protection by vaccination with partially attenuated serotype 1 Marek's disease viruses. Avian Diseases. 2002. v. 46. p. 925-937.

Witter, R.L. Susceptibility of adult chickens to Marek's disease. Proceedings of Western Poultry Disease Conference. 2002. p. 127-128.

Gimeno, I.M., Witter, R.L. and Neumann, U. Neuropathotyping: a new system to classify Marek's disease virus. Avian Diseases. 2002. v. 46. p. 909-918.

Reddy, S.M., Lupiani, B., Gimeno, I.M., Silva, R.F., Lee, L.F. and Witter, R.L. Rescue of a pathogenic Marek's disease virus with overlapping cosmid DNAs: Use of a pp38 mutant to validate the technology for the study of gene function. Proceedings National Academy of Sciences. 2002. v. 99. p.7054 -7059.

Witter, R.L., Schat, K.A. Marek's Disease. Saif, Y.M., Barnes, J., Fadly, A., Glisson, J., McDougal, L., Swayne, D., Editors. Iowa State University Press, Ames, IA. Diseases of Poultry. 2003. p. 407-465.

Borenshtain, R., Witter, R.L., Davidson, I. Persistence of chicken herpesvirus and retroviral chimeric molecules upon invVivo passage. Avian Disease. 2003. v 47. p. 57-59.

Silva, R.F., Reddy, S.M. and Lupiani, B. Expansion of a unique region in the Marek's disease virus genome occurs concomitantly with attenuation but is not sufficient to cause attenuation. Journal of Virology. 2004. v. 78. p. 733-740.

Avian Disease and Oncology Laboratory

Project Title: Epidemiology, Diagnosis and Control of Retrovirus Infection in Poultry
CRIS Number: 3635-32000-013-00D
Scientists: Fadly, A., Silva, R., Hunt, H.,
Location: East Lansing, MI, Avian Disease and Oncology Research
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PROJECT OBJECTIVES:

Avian leukosis virus (ALV) and reticuloendotheliosis virus (REV) are the most common naturally occurring retroviruses associated with neoplastic (cancer-like) diseases in poultry. In addition to causing tumors and other production problems, both ALV and REV are potential contaminants of live-virus vaccines of poultry. Control of retroviruses in poultry is complicated by lack of specific diagnostics and vaccines as well as a high frequency of antigenic and molecular variation among strains of virus. In recent years, the broiler breeder industry has identified an ALV-induced disease termed myeloid leukosis as its highest disease priority. This disease has the potential to negatively influence the economic viability of the entire broiler industry. On the other hand, the principal economic concerns of REV infection are as contaminants of biologic products produced in chicken embryo cells or tissues or as a barrier to export of breeding stock to certain countries. However, sporadic outbreaks of neoplastic disease have been observed in turkeys and infected breeders can, in some cases, transmit the virus to progeny. Understanding the basic mechanisms involved in virus-host interaction, transmission of virus as well as the development of improved diagnostic technology for viruses and tumors are integral components of any successful program for control of retrovirus infection in poultry. The research emphasizes studies on influence of virus-, host-, and environment- related factors on retrovirus-induced diseases in poultry. Using current information on molecular and antigenic characteristics of the virus, more sensitive and specific procedures for detection of virus and antibody will be developed. Currently available technology will also be explored to develop vaccines for use as an adjunct to eradication programs, the principal means for controlling retrovirus infection in poultry. The research will provide the necessary tools for better diagnosis and control of this economically important virus infection of retrovirus infection in poultry.

OVERALL PROJECT ACCOMPLISHMENTS:

This project was initiated in fiscal year 2002. During the last five years (the life of the previous project), infection with subgroup J ALV (ALV-J) and tumors previously thought to be not present in flocks in the U.S. were diagnosed in broiler breeder flocks in various regions of the U.S. Two new tools were developed that aided in the diagnosis of ALV-J: 1) a PCR test (a DNA-based test); and 2) a genetically engineered cell line that is resistant to infection with ALV-J (C/J). Various industry and other diagnostic laboratories have successfully used these new tools in the diagnosis of ALV-J infection in field samples. Also, the envelope gene of the U.S. prototype of ALV-J termed ADOL-Hc1 was identified, sequenced and used as an excellent source of antigen (protein) that is now being used in commercial diagnostic kits for detection of antibody to ALV-J. Scientists at ADOL were first to isolate natural recombinant ALVs with an envelope of subgroup B and LTR of subgroup J (ALV-B/J) from commercial layers flocks suffering from myeloid leukosis. This confirmed that: a) natural recombination between two subgroups of ALV can occur, and b) a recombinant ALV-J was associated with an outbreak of myeloid leukosis in commercial layer flocks; commercial layers are thought to be relatively resistant to ALV-J-induced myeloid leukosis. Preliminary data suggest an influence of age at exposure of embryos and dose of virus on the development of ALV tolerant infection (consistent viremia and lack of antibody).

IMPACT:
Research on avian retroviruses at ADOL, particularly avian leukosis viruses has contributed significantly to the success of programs, currently used by most if not all of primary poultry breeder companies, to reduce or eradicated this important virus infection from their breeding stocks. As vaccines to control avian retroviruses are not available, eradication of virus infection at the primary breeder level is the method of choice for controlling this economically important disease. Successful control of retrovirus infection will reduce economic losses associated with retrovirus-induced tumor mortality and lower productivity. Therefore, successful control of retrovirus infection in poultry will also insure the viability of the United States poultry industry and will also insure a supply of inexpensive meat and egg products of high quality for consumers in the U.S. and in other countries

TECHNOLOGY TRANSFER:
Important new tools were developed that aided industry and diagnostic laboratories in the diagnosis of ALV: 1) a polymerase chain reaction (PCR) test (a DNA-based test); 2) a genetically engineered cell line that is resistant to infection with ALV-J (C/J), and 3) a set of monoclonal antibodies. Also, the envelope gene of the U.S. prototype of ALV-J termed ADOL-Hc1 was identified, sequenced and used as an excellent source of antigen (protein) that is now being used in commercial diagnostic kits for detection of antibody to ALV-J. Most recently, Scientists at ADOL transferred to their counterparts at USDA-APHIS-CVB and industry the protocol used at ADOL for detection of ALV in vaccines and other samples.

PUBLICATIONS:

Silva, R.F., Fadly, A.M., and Hunt, H.D. Hypervariability in the envelope genes of subgroup J avian leukosis viruses obtained from different farms in the U.S. Virology . 2000. v. 272. p. 106-111

Silva, R.F. and A.M. Fadly. Evolution of AVL-J strains. Proceedings International Symposium on ALV-J and Other Retroviruses. . 2000. p. 23-31

Williams, S.M., W.M. Reed, and A.M. Fadly. Influence of age of exposure on the response of line0 and line 6_3 chickens to infection with subgroup J avian leukosis virus. Proceedings International Symposium on ALV-J and Other Retroviruses. 2000. p. 67-76.

Witter, R.L. Determinants of early transmission of ALV-J in commercial broiler breeder chickens. Proceedings International Symposium on ALV-J and Other Retroviruses. 2000. p. 216-225.

Witter, R.L., Bacon, L.D. , Hunt, H.D, Silva, R.F., and Fadly, A.M. Avian leukosis virus subgroup J infection profiles in broiler breeder chickens: association with virus transmission to progeny. Avian Diseases. 2000. v. 44. p. 913-931.

Neumann, U., Berrocal, A., and Fadly, A.M. Morphological evidence for avian leukosis virus (ALV) subgroup J avian myelocytomatosis observed in broiler parent flocks in Costa Rica. Proceedings International Symposium on ALV-J and Other Retroviruses 2000. p. 181-183.

Lupiani, B., Hunt, H.D, Silva, R.F., and Fadly, A.M.. Identification and characterization of recombinant subgroup J avian leukosis viruses (ALV) expressing subgroup A ALV envelope. Virology. 2000. v. 276. p. 37-43

Fadly, A.M. Isolation and identification of avian leukosis viruses: a review. Avian Pathology. 2000. V. 29. p. 529-535.

Fadly, A.M., R.F. Silva, and L.F. Lee. Antigenic characterization of selected field isolates of subgroup J Avian Leukosis Virus. Proceedings International Symposium on ALV-J and Other Retroviruses. 2000. p. 13-22.

Goodwin, M.A., Krushinskie, E.A., Brown, J., Smith, ,E. and Fadly, A.M.. Broilers from parents that have subgroup-J avian leukosis/sarcoma virus tumors are not as economical to produce as broilers from parents that do not have tumors. Proceedings of the 48th Western Poultry Disease Conference. 2000. p. 96.

Hunt, H.D., Lupiani, B. and Fadly, A.M. Recombination between ALV-J and edogenous subgroup E viruses. Proceedings International Symposium on ALV-J and Other Retroviruses. 2000. p. 50-60.

Hunt, H.D., Leathers, V, Lamichhane, C. M., Rosales, G., Jensen, E., McKay, J ., and Fadly, A. M.. Analysis of serum antibody to ALV-J in commercial broilers: Correlation etween ELISA, flow cytometry and virus neutralization. Proceedings International Symposium on ALV-J and Other Retroviruses. 2000. p. 127-140.

Lee, L. F., Fadly, A. M. and Hunt, H. D. Avian leukosis virus subgroup J envelope gene product for diagnosis and immunogenic composition. United States Patent. 2000. #6,146,641.

Witter, R.L., Fadly, A.M. Reduction of horizontal transmission of avian leukosis virus subgroup J in broiler breeder chickens hatched and reared in small groups. Avian Pathology. 2001. V. 30. p. 641-654.

Quin, A., Cui, Z., Lee, L.F., Fadly, A.M. Cloning and expression of envelope gene of subgroup J avian leukosis virus. Chinese Journal of Virology. 2001. v. 17. p. 54-59.

Quin, A., Cui, Z., Lee, L.F., Fadly, A.M. Cloning and sequence of envelope gene of subgroup J avian leukosis virus. Virologica Sinica. 2001. v. 16. p. 68-73.

Williams, S.M. Immunopathogenesis of avian leukosis virus subgroup J in white leghorn chickens. Ph.D. Dissertation. 2001. Michigan State University. 194 p.

Fadly, A.M. An overview of avian retrovirus infections in chickens with special reference to subgroup J avian leukosis virus. Proceedings of XII International Congress of the World Veterinary Poultry Association. 2001. p. 16-22.

Suedmeyer, K., Witter, R.L., and Bermudez, A. Hemangiosarcoma in a golden pheasant (Chrysolophus pictus). J. Avian Medicine and Surgery. 2001. v. 15. p. 126-130.

Qin, A., Lee L.F., Fadly A.M, Hunt, H., and Cui,Z.. Development and characterization of monoclonal antibodies to subgroup J avian leukosis virus. Avian Diseases. 2001. v. 45. p.938-945.

Hussain, A.I., Shanmugam, V., Switzer, W.M., Tsang, S.X., Fadly, Thea A.M., Helfand, D., R., Bellini , W.J., T.M. Folks, and Heneine, W. Lack of evidence of endogenous avian leukosis virus and endogenous avian retrovirus transmission to measles, mumps, and rubella vaccine recipients. Emerging Infectious Diseases 7:66-72. 2001.

Yondem, B.B. Influence of Marek's disease virus on subgroup J avian leukosis virus infection in meat-type chickens. M.S. Dissertation. 2002. Michigan State University. 42 p.

Trampel, D.W., T.M. Pepper, and R.L. Witter. Reticuloendotheliosis in Hungarian partridge. J. Wildlife Diseases. 2002. v. 38. p. 438-442.

Gingerich, E., Porter, R.E., Lupiani, B., Fadly, A.M. Diagnosis of myeloid leukosis induced by a recombinant avian leukosis virus in commercial white leghorn egg laying flocks. Avian Diseases. 2002. v. 46. p. 745-748.

Sung, H.W., Reddy, S.M., Fadly, A.M. High virus titer in feather pulp of chickens infected with subgroup J avian leukosis virus. Avian Diseases. 2002. v. 46. p. 281-286.

Crespo, R., Woolcock, P. R., Fadly, A. M., Hall, C. and Shivaprasad, H. L. Characterization of T-cell lymphomas associated with an outbreak of reticuloendotheliosis in turkeys. Avian Pathology. 2002. v. 31. p. 355-361.

Lupiani, B., Williams, S.M., Silva, R.F., Hunt, H.D., Fadly, A.M. Pathogenicity of Two Recombinant Avian Leukosis Viruses. Avian diseases. 2003. v. 47. p. 425-432.

Garcia, M., Narrang, N., Reed, W. M. and Fadly, A. M. Molecular Characterization of reticuloendotheliosis virus (REV) insertion in the genome of field and vaccine strains of fowlpox virus(FPV). Avian Diseases. 2003. v. 47. p. 343-354.

Fadly, A. Neoplastic Diseases of Poultry: Introduction. Saif, M., Barnes, J., Fadly, A., Glisson, J., McDougal, L., Swayne, D., Editors. Iowa State University Press, Ames, IA. Diseases of Poultry. 2003. p. 405-407.

Fadly, A., Payne, L. Leukosis and Sarcoma Group. Saif, M., Barnes, J., Fadly, A., Glisson, J., McDougal, L., Swayne, D., Editors. Iowa State University Press, Ames, IA. Diseases of Poultry. 2003. p. 465-516.

Witter, R.L., Fadly, A.M. Reticuloendotheliosis. Saif, M., Barnes, J., Fadly, A., Glisson, J., McDougal, L., Swayne, D., Editors. Iowa State University Press, Ames, IA. Diseases of Poultry. 2003. p. 517-536.

Williams, S.M., Reed, W.M., Bacon, L.D. and Fadly, A.M.. Response of white leghorn chickens of various genetic lines to infection with avian leukosis virus subgroup J. Avian Diseases. 2004. v.48. p.61-67.

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PROJECTS

Foodborne Contaminants Research
- Development of New Technologies for Detection of Prions in Animal Feed and Environmental Samples

Foodborne Contaminants Research

Project Title: Development of New Technologies for Detection of Prions in Animal Feed and Environmental Samples
CRIS Number: 5325-32000-003-00D
Scientists: M. Carter, R. Wong (30%), Vacant, Vacant
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PROJECT OBJECTIVES:

The primary focus of this CRIS is investigation and development of novel technologies for detection, quantitation, and strain characterization of both normal and abnormally-folded prion proteins. The methods investigated are suitable for use with environmental samples as well as animal feed and feed additives. Because the food industry will not tolerate false positive or false negative test results, the new detection methods must be highly sensitive and specific. In addition, they must be rapid, cost effect, and easily interpreted. Major goals include 1) significant improvements in sensitivity for prion assays, including methods for strain-specific detection. In addition to immunochemistry and traditional analytical chemistry, hybrid methods combining the best features of both approaches are being investigated. Strain-specific detection is likely to require further orthogonal assay technologies. Additional goals include 2) effective sampling methods for capturing, concentrating, and detecting prions, other animal proteins, and surrogates from samples of air, water, soil, feed, and feed additives; and 3) development of inactivation/destruction of prions for decontamination. The methods investigated must be appropriate for sampling and decontamination of food processing surfaces and animal holding pens. Thus they must be: free from chemical or physical extremes; safe, with no generally toxic residue; and relatively inexpensive, for use on large areas. The objectives of this CRIS overlap somewhat with those of CRIS 5325-42000-027-00D, Development of New Technologies for Rapid Identification of Pathogen/Pathogen Products.

OVERALL PROJECT ACCOMPLISHMENTS:

- A method for detecting meat and bone in animal feed has been developed based on a GC/MS system that detects the presence of cholesterol. A linear response from 0.5 ng (~ 2 picomole) to 60 ng (~ 150 picomole) is observed. This method can be used to detect MBM contamination at levels between 0.1 and 5 %. The assay is being converted into a rapid, field portable immunoassay that can be applied to feed or meal samples.
- Significant advances have been made in the development of a mass spectrometric method for quantitation of prions at low levels. Model peptides similar to those expected from PrP^{Sc} digests have been analyzed by linear ion trap technology using nanoLC and electrospray technology. Limits of detection in the low attomolar level have been obtained. If this can be achieved with PrP^{Sc} peptides, an assay that is several orders of magnitude more sensitive than extant antibody-based methods should be possible. Digests of hamster prions have been completed and analyzed by qualitative mass spectrometry to identify peptide targets for quantitative work in the future. A patent disclosure has been written which has been approved for patent filing within the next 6 months.
- New monoclonal antibodies that bind PrP have been developed by immunization of PrP ablated mice and a modified cell fusion protocol. These antibodies have broad specificity and extend the detection range for PrP^{Sc} in a Conformation Dependent Immunoassay.

- In an effort to produce better PrP antibodies, PrP ablated BALB/c mice and PrP ablated BALB/c mouse derived myeloma cells are being produced. PrP ablated animals with greater than 90% BALB/c background already have been produced and myeloma induction is underway.

IMPACT:
More sensitive tests for prions are essential in order to develop a live-animal test. Such tests would greatly simplify cattle management for BSE and management of cervid populations for CWD. These assays will extend our ability to track prions in environmental samples, and they will be useful tools for evaluating prion inactivation methods, and for pathogenesis studies.

TECHNOLOGY TRANSFER:
The mass spectrometric method for detecting prions and prion strains has been approved for a patent application. Once issued, this approach can be licensed, making the method readily available to producers and regulators.

PUBLICATIONS:

None

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PROJECTS

Animal Diseases Research Unit

- Anaplasmosis: A Genomic Approach to Vaccine Development
- On Farm Ecology of *Listeria Monocytogenes*
- Biology of Ruminant Gammaherpesviruses Associated with Malignant Catarrhal Fever
- Ovine Prion and Viral Infections: Scrapie and Ovine Progressive Pneumonia, Diagnosis and Control
- Functional Approaches to the Control of Babesiosis in Cattle and Horses
- BSE and Other TSEs Ruminant Herbivors: Diagnosis, Transmission and Genetics

Animal Diseases Research Unit

Project Title: Anaplasmosis: A Genomic Approach to Vaccine Development
CRIS Number: 5348-32000-016-00D
Scientists: Scoles, G., Suarez, C., Goff, W., Knowles, D.
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PROJECT OBJECTIVES:
Anaplasmosis is one of the most prevalent arthropod-borne hemoparasitic diseases that continue to constrain the production, movement and utilization of cattle worldwide. Extensive losses continue within the major cattle producing regions of the world. The economic losses occur through disease and export-import restrictions. Immunization against the causative rickettsial pathogen, *Anaplasma marginale*, with a safe and effective vaccine is not yet feasible on a practical basis. There are three main research objectives. These objectives are the development of accurate method(s) of diagnosis, the development of a safe and efficacious vaccine and acquisition of a detailed understanding of vector transmission with a goal of improved control methods. We have completed the first objective. Our approach to vaccine development (objective 2) has three components. The first component is dissection of the bovine immune response, which develops in response to infection and is protective in the chronic (persistent) phase. The second component is dissection of the interface between *Anaplasma marginale* and the tick vectors, which transmit this organism. This component has the unique advantage of identifying components of the organism, which are exposed only during development in the vector and therefore may be targets for vaccine development. Our final component, completed within this past year is genomic sequencing and annotation of the *A. marginale* genome.

OVERALL PROJECT ACCOMPLISHMENTS:
The development and commercialization of our diagnostic test for Anaplasma marginale infection is completed. This test is now commercially available and has been adopted by Canada as their official test. This test will have major impact internationally on the control of anaplasmosis. This test will allow, for the first time, accurate epidemiological studies to be conducted. During recent months we've complete a collaborative project with CEAH, APHIS in conducting a serologic survey, using our cELISA, for detection of *A. marginale* infection in cattle within the United States. Also, producers will be able to test and segregate infected cattle in areas in which ticks are present, or adopt new management tools in handling infected cattle, such as assuring the same needle isn't used to process both infected and uninfected cattle. Accurate diagnosis will now allow for the possibility of opening export markets to countries which were previously thought to be free of anaplasmosis, but upon testing with accurate methodology are found to have infected cattle, sheep or goats. This recently occurred in Canada when through collaboration with our Unit it was discovered that bison within Canada are infected with *A. marginale*.

IMPACT:
The competitive ELISA for identification of cattle persistently infected with *Anaplasma marginale* has been transferred to industry and its final approval for distribution as a kit was approved in 2002. We provide to APHIS on continual basis information to aid in vector control at the southern U.S. border. We have also aided Canada in providing diagnostic expertise in a recent discovery of *Anaplasma marginale* infection in bison within Canada. This diagnostic kit was used in 2003 for congressional budget meetings. Most importantly our diagnostic test has been used by APHIS to begin establishing the epidemiology of *A. marginale* within the

United States. These data are being used in adjusting current *A. marginale* import restrictions placed on U. S. cattle by Canada.

TECHNOLOGY TRANSFER:
A competitive ELISA was licensed and transferred to industry. This test is commercially available (U.S. Veterinary License No. 332) and has been adopted by Canada as their official import test.

PUBLICATIONS:

Davidson, W.R., Goff, W.L. Anaplasmosis. In: Infectious Diseases of Wild Mammals, 3rd edition (E. Williams and I Barker, eds.) Iowa State University Press. 2001. p. 455-466.

Futse, J.E., Massaro, U.W., Knowles, D.P., Palmer, G.H. Transmission of Anaplasma marginale by Boophilus microplus: Retention of vector competence in the absence of vector-pathogen interaction. Journal of Clincal Microbiology. 2002. v. 41. p. 3829-3834.

Scoles, G.A. Phylogenetic analysis of the Francisella-like endosymbionts of Dermacentor Ticks. Journal of Medical Entomology. 2004. v. 41 (in press).

Gorham, J., and Knowles, D. 2000. Biotechnology in the diagnosis of infectious diseases and vaccine development. OIE Manual of Standards for diagnostic tests and standards: biotechnology, 1.7.

Bradway, D. S., Torioni de Echaide, S., Knowles, D. P., Hennager, S. G., and McElwain, T. F. 2000. Sensitivity and specificity of the complement fixation test for detection of *Anaplasma marginale* persistently infected cattle. Journal of Veterinary Diagnostic Investigation. 13:77-79.

Valdez, R. A., McGuire, T. C., Brown, W. C., Davis, W. C., and Knowles, D. P. 2001. Long-term in vivo depletion of functional CD4+ T lymphocytes from calves requires both thymectomy and anti-CD4 monoclonal antibody treatment. Immunology, 102:426-433.

Ferreira, A. M. T., Suzart, S., Vidotto, O., Knowles, D. P., and Vidotto, M. C. 2001. Use of repetitive DNA elements to define genetic relationships among *Anaplasma marginale* isolates FEMS Microbiology Letters, 197:139-143.

Brayton, K. A., Knowles, D. P., McGuire, T. C., and Palmer, G. H. 2001. Efficient use of a small genome to generate antigenic diversity in tick-borne ehrlichial pathogens. Proceedings National Academy of Science, USA, 98:4130-4135.

Valdez, R. A., McGuire, T. C., Brown, W. C., Davis, W. C., Jordan, J. M., and Knowles, D. P. 2002. Selective in vivo depletion of CD4+ T lymphocytes with anti-CD4 monoclonal antibody during acute infection of calves with *Anaplasma marginale*. Clinical and Diagnostic Laboratory Immunology. 9:417-424.

Animal Disease Research Unit

Project Title: On farm Ecology of *Listeria Monocytogenes*
CRIS Number: 5348-32000-022-00D
Scientists: Borucki, M., Knowles, D.P.
Location: Pullman, WA, Animal Diseases Research Unit
Contact: Borucki, M., Phone: (509) 335-7407, Fax: (509) 335-8328, mborucki@vetmed.wsu.edu

PROJECT OBJECTIVES:

Listeria monocytogenes is a bacterium capable of causing serious disease in humans and animals. *L. monocytogenes* infection of cattle and sheep can lead to disease of the central nervous system and death. Human listeriosis is a potentially fatal food borne disease often associated with the consumption of contaminated dairy products. Because *L. monocytogenes* is widely distributed in the environment, a major problem associated with the food safety issue is the source(s) of *L. monocytogenes* in food borne illness. The role of domestic animals in dairy product contamination is not clearly understood. The focus of our research program is to elucidate the sources and significance of dairy farm contamination as related to human and animal disease. A majority of human outbreaks are caused by just 3 of the 13 *L. monocytogenes* serotypes, therefore we will be focusing on identifying the prevalence of these serotypes on the dairy farm and developing methods to precisely and reliably identify genetic subtypes that are especially virulent. Specifically, our project has three major objectives: (1) characterize on-farm subtypes of livestock origin and compare these subtypes to those of food-borne disease origin; (2) develop a rapid and informative assay to identify *L. monocytogenes* serotypes, subtypes, and identify the genes that differ between epidemic and nonepidemic strains; (3) identify or develop an in vivo or in vitro model capable of quantifying the virulence potential of *L. monocytogenes* strains.

OVERALL PROJECT ACCOMPLISHMENTS:

Objective 1: Determine the prevalence and subtypes of *L. monocytogenes* present on Pacific Northwest dairy farms. Approximately 775 raw milk samples from 474 Pacific Northwest herds were tested at multiple timepoints and isolates obtained from the milk samples were serotyped, genetically characterized, and evaluated for unique biological properties. A vast majority of *L. monocytogenes* isolates obtained from the milk and farm samples were found to be clinically significant serotypes that persist in the dairy environment for an extended period of time. Research was also done to investigate whether human listeriosis cases are associated with the same subtypes of *L. monocytogenes* found in raw milk and area dairy farms by comparing farm-associated subtypes to human isolates. Several human isolates were genetically similar or identical to farm and milk strains and it was also determined that a strain responsible for a large 1985 epidemic has a current, local on-farm reservoir and continues to cause sporadic human disease. This work was an important step in defining the on-farm ecology of *L. monocytogenes* and accessing the contribution of farm contamination as a source in human listeriosis.

Objective 2: Construct and test a DNA microarray to allow rapid, accurate and discriminatory subtyping of *L. monocytogenes* isolates for the purpose of on farm molecular epidemiology and gene discovery. Research was done to test the utility of a mixed genome DNA microarray analysis for genetic subtyping of *L. monocytogenes* strains and to identify the genetic differences between subtypes. Fifty *L.*

monocytogenes isolates were subtyped using a 585-probe mixed genome microarray and genes that differentiate the major genetic divisions were identified. Microarray analysis results preserved previously described phylogenetic relationships between major serogroups, discriminated among isolates within a serotype, and identified specific genes that vary between isolates including several putative virulence factors. Identification of serotype-specific regions allowed the development of PCR primers for rapid, accurate, and inexpensive identification of *L. monocytogenes* serotypes.

To further investigate genetic regions that characterize subtypes, a subsequent 2000-probe mixed genome DNA microarray was constructed and used to identify 675 polymorphic (informative) probes for sequence analysis and construction of a condensed microarray. Fifty-five *L. monocytogenes* strains were genetically characterized using the condensed array including strains that had been subtyped previously by Dr. Franco Pagotta (Health Canada) and Dr. Sandra Smole (MA State Laboratory Institute) using pulsed-field gel electrophoresis (PFGE), ribotyping, and multilocus sequence typing (MLST). Microarray subtyping had resolution equal to that of PFGE (using *Asc*I and *Apa*I digests), higher resolution than ribotyping and MLST (using 8 housekeeping genes), and correctly grouped eleven out of twelve isolates that had an epidemiological link. Additionally, a majority of epidemic strains were grouped together within phylogenetic Division I. Discriminant function analysis allowed identification of 22 probes from the mixed-genome array that distinguish serotypes and subtypes including several potential markers that were distinct for the epidemic cluster. Many of the subtype-specific genes encode proteins that likely confer survival advantage in the environment and/or host.

Objective 3: Develop in vitro and in vivo virulence models for *L. monocytogenes* infection that allow the phenotypic characterization of putative virulence genes identified by microarray analysis. In order to determine if a particular subtyping method is effectively able to differentiate virulent isolates from less virulent "environmental" isolates one must first be able to access the virulence potential of a particular *L. monocytogenes* strain. Isolates known to be associated with human or animal clinical cases are known to be virulent. However, isolates found in the environment may also be virulent given the right host and dose. Therefore, it is important to develop an assay that is able to predict the virulence potential of environmental/nonclinical strains. Six human epidemic strains and six environmental strains were assayed for invasiveness using a modified intragastric inoculation A/J mouse model (Czuprynski et al., 2003). Variation in strain invasiveness was observed and epidemic strains were significantly more invasive than environmental strains (P=0.01). A/J strain mice purchased from one vendor were significantly more susceptible than A/J mice purchased from a different vendor.

IMPACT:

The prevalence of *L. monocytogenes* contamination in Pacific Northwest area bulk milk tanks was previously unknown. The results from Objective 1 are an important initial step in defining the on-farm ecology of *L. monocytogenes*, the regional distribution of *L. monocytogenes* subtypes, and accessing the contribution of farm contamination as a source in human listeriosis. Results from Objective 2 demonstrate the applicability of microarray analysis to *L. monocytogenes* subtyping and gene discovery. These results show that a relatively simple microarray had resolution comparable to, or exceeding, existing methods and correctly identified strains associated with food poisoning outbreaks. Importantly, this method also identified the genetic regions that differ between strains and thus identified numerous genetic markers that are currently being used in epidemiological and pathogenesis studies. This microarray data also allowed development of alternative genetic methods to serotype isolates quickly and inexpensively. An intragastric inoculation mouse model was

developed (Objective 3) that demonstrated that epidemic strains as a group are more infective than environmental strains. The ability to accurately assess the disease-causing potential of an isolate is essential for defining the genes that control strain virulence. Development of this model is a critical step towards understanding why some strains cause epidemics whereas others do not.

TECHNOLOGY TRANSFER:

PUBLICATIONS:

Borucki, M.K., Krug, M.J., Muraoka, W.T., Call, D.R. Discrimination among *Listeria monocytogenes* isolates using a mixed genome DNA microarrays. Veterinary Microbiology. 2003. v. 92. p. 351-362.

Call, D.R., Borucki, M.K., Besser, T E. Mixed-genome microarrays reveal multiple serotype and lineage-specific differences among strains of *Listeria monocytogenes*. Journal of Clinical Microbiology. 2003. v. 41. p. 632-639.

Call, D.R., Borucki, M.K., Loge, F.J. Detection of bacterial pathogens in environmental samples using DNA microarrays. Journal of Microbiological Methods. 2003. v. 53. p. 235-243.

Palumbo, J.D., Borucki, M.K., Mandrell, R.E., Gorski, L.A. Serotyping of *Listeria monocytogenes* by ELISA and identification of mixed-serotype cultures by colony blot. Journal of Clinical Microbiology. 2003. v. 41. p. 564-571.

Muraoka, W.T., Gay, C., Knowles, D., Borucki, M.K. Prevalence of *Listeria monocytogenes* in bulk milk of the Pacific Northwest. Journal of Food Protection. 2003. v. 66. p. 1413-1419.

Borucki, M.K., Peppin, J.D., White, D., Loge, F., Call, D.R. Variation in biofilm formation among strains of *Listeria monocytogenes*. Applied Environmental Microbiology. 2003. v. 69. p. 7336-7342.

Borucki, M.K., Call, D.R. *Listeria monocytogenes* serotype identification by PCR. Journal of Clinical Microbiology. 2003. v. 41. p. 5537-5540.

Wesley I, Borucki M, Call D.R, Larson D, Schroeder-Tucker L. Detection and diagnosis of *Listeria monocytogenes* and listeriosis in animals. In: Torrence ME, Isaacson RE, eds. Microbial Food Safety in Animal Agriculture. Ames: Iowa State Press. 2003 p. 233-242.

Kim, S.H., Bakko, M., Knowles, D., Borucki, M.K. Oral inoculation of A/J mice detects invasiveness differences between *Listeria monocytogenes* epidemic and environmental strains. Infection and Immunity. (In press).

Animal Disease Research Unit

Project Title: Biology of Ruminant Gammaherpesviruses Associated with Malignant Catarrhal Fever
CRIS Number: 5348-32000-018-00D
Scientists: Li, H., Taus, N.S., Knowles Jr, D.P.
Location: Pullman, WA, Animal Diseases Research Unit
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PROJECT OBJECTIVES:
Malignant catarrhal fever (MCF) is a significant source of economic loss in several major ruminant species, including cattle, bison, and deer. In most of the world outside of Africa, sheep are the major source of the virus that causes the syndrome, referred to in this instance as sheep-associated MCF. Diagnosis of MCF, particularly sheep-associated MCF, historically has been problematic. The major goals for this project are to develop new methods for effective diagnosis and control of MCF. The program has resulted in several reliable diagnostic assays such as competitive inhibition ELISA and quantitative fluid-phase PCR. Using these newly developed assays, we have been able to construct a reasonable picture of the life cycle of the virus in its natural host and to develop control programs to prevent transmission.

OVERALL PROJECT ACCOMPLISHMENTS:
Over the past few years this project has brought about fundamental and dramatic advances in the understanding of MCF and in the preferred methodologies used for diagnoses and control. The development of the competitive inhibition ELISA has been the single most important accomplishment, as it allowed reliable serological diagnosis of infection, thereby enabling effective investigations into the epidemiology of the virus. We have also applied the PCR test for sheep-associated MCF virus DNA to these same questions. The combination of the two technologies, aggressively applied to the problems, has enabled us to develop a reasonably comprehensive picture of the life cycle of this group of viruses in nature. Recently we intensively examined viral shedding patterns from 6 to 9 month-old adolescent and adult sheep, showing that nasal shedding is the major mode for viral transmission, and adolescents are the highest risk group for viral shedding. Once we understood the transmission patterns among sheep, we were able to clear up much of the mystery traditionally associated with MCF epidemiology, to design a management-based control program to prevent transmission, and to produce MCF virus-free sheep. We have also developed a competitive PCR and adapted a real-time PCR for the quantitation of sheep-associated virus DNA. These assays should prove instrumental in defining the temporal distribution of virus in the host, and the time-specific shedding patterns of both carriers and affected hosts. A PCR assay for sheep-associated MCF was adapted for use on DNA extracted from formalin-fixed paraffin-embedded tissues, which enables retrospective examination of specimens for the virus. An in situ PCR was also developed for detection of sheep-associated virus in MCF-affected animals, which is in the early stages of application to pathogenesis studies. Recently a new member of the MCF virus group capable of causing classic MCF in white-tailed deer was identified. In addition, we also discovered that goats are endemically infected with their own strain of MCF virus that is closely related to, but distinct from, the classic sheep strain of virus and showed it to be capable of causing disease in deer that presents significantly differently from the classic MCF. These findings illustrate the complexity of the life cycles of members of the MCF virus group in nature and provide additional information to guide diagnosis and development of control measures.

IMPACT:
Development of an accurate and practical monoclonal antibody-based MCF serological test and establishment of various PCR assays specific for MCF viruses have significantly improved MCF diagnostics, and have provided essential tools for MCF research. Our additional accomplishments in epidemiological studies represent a very significant milestone in the history of MCF investigation. Despite many studies by many investigators over the past 60 to 70 years, the etiology and epidemiology of sheep-associated MCF have remained a controversial enigma. Our studies provide a basis for understanding the intriguing biological events that occur with this virus, and enable rational explanations for the previously enigmatic and confusing observations concerning transmission. Production of virus-free sheep that pose no threat to their MCF-susceptible neighbours could be a great benefit to limited-size cattle-sheep operations and bison owners, as well as petting zoos and game farms. The studies highlight how to effectively avoid severe MCF losses in clinically susceptible species, particularly in bison.

TECHNOLOGY TRANSFER:
The competitive ELISA for detection of MCF viral antibody against an epitope that is conserved among MCF group viruses in ruminant species has been transferred to industry (VMRD, Inc, Pullman) and the kit has been distributed internationally since 2002. Four additional monoclonal antibodies against MCF virus have also been transferred to VMRD, Inc.

A diagnostic service, consisting of cELISA and various PCRs, for MCF has been established through the Washington Animal Disease Diagnostic Laboratory. We are now considered the reference laboratory of choice for MCF PCR and serology.

PUBLICATIONS:

Li, H., Snowder, G., O'Toole, D., Crawford, T.B. Transmission of ovine herpesvirus 2 among adult sheep. Veterinary Microbiology. 2000. v. 71. p. 27-35.

Li, H., Dyer, N., Keller, J., Crawford, T.B. Newly recognized herpesvirus causing malignant catarrhal fever in white-tailed deer (*Odocoileus virginianus*). Journal of Clinical Microbiology. 2000. v. 38. p. 1313-1318.

Li, H., Keller, J., Knowles, D.P., Crawford T.B. Recognition of another member of the malignant catarrhal fever (MCF) virus group: an endemic gammaherpesvirus in domestic goats. Journal of General Virology. 2001. v. 82. p. 227-232.

Li, H., Hua, Y., Snowder, G., Crawford T.B. Levels of malignant catarrhal fever viral DNA in nasal secretions and blood of sheep: implication for transmission. Veterinary Microbiology. 2001. v. 79. p. 301-310.

Muller-Doblies, U.U., Egli, J., Li, H., Braun, U., Ackermann, M. Malignant catarrhal fever in Switzerland, part 1: epidemiology. Swiss Archives of Veterinary Medicine. 2001. v. 143. p. 173-183.

Li, H., McGuire, T.C., Muller-Doblies, U.U., Crawford, T.B. A simpler, more sensitive competitive inhibition enzyme-linked immunosorbent assay for detection of antibody to malignant catarrhal fever viruses. Journal of Veterinary Diagnostic Investigation. 2001. v. 13. p. 361-364.

Müller-Doblies, U.U., Egli, J., Hauser, B., Li, H., Strasser, M., Ehrensperger, F., Braun, U., Ackermann, M. Malignant catarrhal fever in Switzerland, part 2: evaluation of diagnostics. Swiss Archives of Veterinary Medicine. 2001. v. 143. P. 581-591.

Li, H., Snowder, G., Crawford, T.B. 2002. Effect of passive transfer of maternal immune components on infection with ovine herpesvirus 2 in lambs. American Journal of Veterinary Research. 2002. V. 63. P. 631-633.

O'Toole, D., Li, H., Sourk, C., Montgomery, D.L., Crawford, T.B. Malignant catarrhal fever in a bison feedlot 1994 – 2000. Journal of Veterinary Diagnostic Investigation. 2002. v. 14. P. 183-193.

Zarnke, R.L., Li, H., Crawford, T.B. Serum antibody prevalence of malignant catarrhal fever viruses in seven wildlife species from Alaska. Journal of Wildlife Diseases. 2002. v. 38. p. 500-504.

Crawford, T.B., Li, H., Rosenberg, S.S., Norhausen, R.W., Garner, M.M. Mural folliculitis and alopecia in Sika deer caused by the goat-associated malignant catarrhal fever virus. Journal of American Veterinary Medical Association. 2002. v. 211. p. 843-847.

Albini, S., Zimmermann, W., Neff, F., Ehlers, B. Hani, H., Li, H., Hüssy, D., Engles, M., Ackermann, M. Identification and quantification of gammaherpesvirus 2 DNA in fresh and stored tissues of pigs with symptoms of porcine malignant catarrhal fever. Journal of Clinical Microbiology. 2003. v. 41. p. 900-904.

Albini, S., Zimmermann, W., Neff, F., Ehlers, B., Häni, H., Li, H., Hüssy, D., Casura, C., Engels, M., Ackermann, M. Diagnostic findings in pigs with porcine malignant catarrhal fever. Swiss Archives of Veterinary Medicine. 2003. v. 145. p. 61-68.

Li, H., Wunschmann, A., Keller, J., Hall, D.G., Crawford, T.B. Caprine herpesvirus-2 associated malignant catarrhal fever in white-tailed deer (*Odocoileus virginianus*). Journal of Veterinary Diagnostic Investigation. 2003. v. 15. p. 46-49.

Simon, S., Li, H., Crawford, T.B., Oaks, L. The vascular lesions of a cow and bison with sheep-associated malignant catarrhal fever contain ovine herpesvirus 2-infected CD8+ T lymphocytes. Journal of General Virology. 2003. v. 84. p. 2009-2013.

Kim, O., Li, H., Crawford, T.B. Demonstration of sheep-associated malignant catarrhal fever virions in sheep nasal secretions. Virus Research. 2003. v. 98. p. 117-122.

Bender, L.C., Li, H., Thompson, B.C., Morrow, P.C., Valdez, R. Infectious disease survey of gemsbok in New Mexico. Journal of Wildlife Diseases. 2003. v. 39. p. 772-778.

Li, H., Gailbreath, K., Bender, L.C., West, K., Keller, J., Crawford, T.B. Evidence of three new members of malignant catarrhal fever virus group in muskox (*Ovibos moschatus*), Nubian ibex (*Capra nubiana*) and gemsbok (*Oryx gazelle*). Journal of Wildlife Diseases. 2003. v. 39. p. 875-880.

Animal Diseases Research Unit

Project Title: Ovine Prion and Viral Infections: Scrapie and Ovine Progressive Pneumonia, Diagnosis and Control
CRIS Number: 5348-32000-019-00D
Scientists: Alverson, J., Herrmann, L., Knowles Jr, D.P., O'Rourke, K.I.
Location: Pullman, WA, Animal Diseases Research Unit
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PROJECT OBJECTIVES:
The economic losses to the sheep industry due to ovine scrapie can be reduced by a coordinated program of live animal testing, replacement with sheep of lower susceptibility, and reducing transmission within the flock. Scrapie is a transmissible spongiform encephalopathy associated with deposition of an abnormal isoform of a mammalian glycoprotein, the prion protein, in tissues throughout the sheep. The highest level of accumulation is in the brain, although detectable levels are found in lymphoid tissues and placenta/fetal tissues. Detection of prions in peripheral lymphoid tissue can be used to identify and cull infected animals early in infection. Further, the susceptibility of sheep to clinical scrapie and to accumulation of prions is under genetic control. Quantitative determination of the level of protection associated with commonly occurring genotypes could enable producers and regulatory programs to integrate protective genetics with elimination of infected stock to reduce the amount of disease in U.S. flocks. Control of all domestic prion diseases is important in reducing trade barriers for U.S. sheep and germplasm and for assuring the present and future global markets for cattle. Ovine progressive pneumonia virus causes persistent viral infection and development of multi-organ inflammatory disease in some animals. The infection rates in US sheep flocks range from 9 to 49%. A variable and unpredictable percentage of infected animals will progress to clinical disease with high viral titers and increased risk of transmission to flockmates. This project has produced a serologic test for the disease. The current project includes development of a vaccine to reduce infected animals and identification of genes associated with decreased transmission in infected sheep.

OVERALL PROJECT ACCOMPLISHMENTS:
A practical live animal test for scrapie and preclinical postmortem tests for scrapie were developed and transferred to the regulatory agencies for use in the US. Monoclonal antibodies useful in assays on routinely formalin fixed tissue from infected sheep, deer, elk, cattle, humans, mink, domestic cats and a wide variety of captive wildlife potentially exposed to prion diseases were developed. Mechanisms for preventing transmission of scrapie through genetic selection of sires were demonstrated. Along with sire testing, genetic testing in bred ewes will ensure less transmission of scrapie from ewe to lamb. A project investigating scrapie prevalence in goats and the importance of goats in the transmission of scrapie to sheep was initiated. We have initiated another research project relating to transmission of OPPV at the Experimental Sheep Station in Dubois, ID. We continue to test a small sheep flock (Future Farmers of America Herd) (<30) for OPPV in Sumner, WA, associated with Sumner High School.

IMPACT:
The development of the third eyelid test for scrapie diagnosis and prevention of scrapie transmission by screening for PrP diploid genotype has directly benefited the sheep industry. In addition, investigations of

different transmission sources namely salivary glands, peripheral blood mononuclear cells, and placental tissues has resulted in an increased understanding regarding the mechanisms of scrapie transmission. Both determination of the sensitivity limit of PrP(Sc) detection in immunohistochemistry and determination of the specific lymphoid cell types that accumulate truncated and full length PrP(Sc) have established the specificity and sensitivity requirements for PrP(Sc) detection and will aid future studies on detection of PrP(Sc) in blood.

Development of a prime-boost vaccine strategy for caprine arthritis encephalitis virus (CAEV) resulted in protection in CAEV challenged goats. Since ovine progressive pneumonia virus (OPPV) and CAEV are considered quasispecies, this finding will guide the future testing of this vaccine on sheep. In addition, the high sensitivity and specificity of the CAEV cELISA in both goats and sheep along with the ability to quantitate the amount of anti-CAEV/OPPV antibody will aid accurate diagnosis and eradication of small ruminant lentiviruses from flocks and herds throughout the United States. The cELISA was used in the National Animal Health Monitoring System (NAHMS) for seroprevalence of OPPV in U.S. sheep flocks which was performed by the Centers of Epidemiology and Animal Health, APHIS, Fort Collins, CO in 2001.

Together, these contributions have reduced economic losses to the sheep industry. The eventual eradication of scrapie and OPPV will reopen U.S. sheep exports which will increase economic gains to the sheep industry.

TECHNOLOGY TRANSFER:

Licenses:

Two monoclonal antibodies to the prion protein and their use in combination as detection reagents for prions have been patented. Both antibodies are commercially available. Only non-exclusive licenses have been offered, to insure the widest possible use of these reagents in diagnostics, industry, and research. The antibodies are in use internationally and collaborative programs to train personnel in Canada, Mexico, and China are in progress. The preclinical test for scrapie has been transferred to the National Veterinary Services Laboratory. APHIS has established a national testing network, through which veterinary and state diagnostic laboratories will apply the technology under contract with APHIS. CAEV cELISA is currently licensed for goats and will be licensed for sheep and commercially available in the U.S. in 6-12 months.

PUBLICATIONS:

Bender, S., Alverson, J., Herrmann, L.M, O'Rourke, K.I. Histamine aids biopsy of third eyelid lymphoid tissue in sheep. Veterinary Record (in press)

Caplazi, P., O'Rourke, K.I, Baszler, T.V. Resistance to scrapie in PrP ARR/ARQ heterozygous sheep is not caused by preferential allelic use. Journal of Clinical Pathology (in press).

Hamir, A.N, Miller, J.M, O'Rourke, K.I, Bartz, J.C, Stack, M.J, Chaplin, M.J. Transmission of transmissible mink encephalopathy to raccoons (Procyon lotor) by intracerebral inoculation. Journal of Veterinary Diagnostic Investigation. 2004. v. 16(1). p. 57-63.

Herrmann, L.M., Hotzel, I., Cheevers, W.P., Pretty On Top, K.P., Lewis, G.S., Knowles, D.P. Seven new ovine progressive pneumonia virus (OPPV) field isolates from Dubois Idaho sheep comprise part of OPPV clade II based on surface envelope glycoprotein (SU) sequences. Virus Research. 2004. v. 102(2) p. 215-20.

Herrmann, L.M., Cheevers, W.P., Davis, W.C., Knowles, D.P., O'Rourke, K.I. CD21-positive follicular dendritic cells a possible source of PrPSc in lymph node macrophages of scrapie-infected sheep. American Journal of Pathology. 2003. v. 162(4). p. 1075-1081.

Herrmann, L.M., Cheevers, W.P., McGuire, T.C., Adams, D.S., Hutton, M.M., Gavin, W.G., Knowles, D.P. Competitive-inhibition enzyme-linked immunosorbent assay for detection of serum antibodies to Caprine Arthritis-Encephalitis Virus: diagnostic tool for successful eradication. Clinical and Diagnostic Laboratory Immunology. 2003. v. 10(2). p. 267-271.

Herrmann, L.M., Cheevers, W.P., Marshall, K.L., McGuire, T.C., Hutton, M.M., Lewis, G.S., Knowles, D.P. Detection of serum antibodies to Ovine Progressive Pneumonia Virus (OPPV) in sheep using a Caprine Arthritis-Encephalitis Virus (CAEV) competitive-inhibition Enzyme-Linked Immunosorbent Assay (cELISA). Clinical and Diagnostic Laboratory Immunology. 2003. v. 10(5). p. 862-865.

Maxson, L., Wong, C., Herrmann, L.M., Caughey, B., Baron, G.S. A solid-phase assay for identification of modulators of prion protein interactions. Analytical Biochemisty. 2003. v. 323(1). p. 54-64.

Paramithiotis, E., Pinard, M., Lawton, T., LaBoissiere, S., Leathers, V.L., Zou, W-Q., Estey, L.A., Lamontagne, J., Lehto, M.T., Kondejewski, L.H., Francoeur, G.P., Papadopoulos, M., Haghighat, A., Spatz, S.J., Head, M., Will, R., Ironside, J., O'Rourke, K., Tonelli, K., Ledebur, H.C., Chakrabartty, A., Cashman, N.R. A prion protein epitope selective for the pathologically misfolded conformation. Nature Medicine. 2003. v. 9(7). p. 893-899.

Valdez, R.A., Rock, M.J., Anderson, A.K., O'Rourke, K.I. Immunohistochemical detection and distribution of prion protein in a goat with natural scrapie. Journal of Veterinary Diagnostic Investigation. 2003. v. 15. p. 157-162.

Cheevers, W.P., Snekvik, K.R., Trujillo, J.D., Kumpula-McWhirter, N.M., Pretty On Top, K.J., Knowles, D.P. Prime-boost vaccination with plasmid DNA encoding caprine-arthritis encephalitis lentivirus env and viral SU suppresses challenge virus and development of arthritis. Virology. 2003. v. 306. p. 116-125.

O'Rourke, K.I., Duncan, J.V., Logan, J.R., Anderson, A.K., Norden, D.K., Williams, E.S., Combs, B.A., Stobart, R.H., Moss, G.E., Sutton, D.L. Active Surveillance for scrapie by third eyelid biopsy and genetic susceptibility testing of flocks of sheep in Wyoming. Clinical and Diagnostic Laboratory Immunology. 2002. v. 9(5). p. 966-971.

Herrmann L.M, Baszler T.V, Knowles D.P, Cheevers W.P. PrP(Sc) is not detected in peripheral blood leukocytes of scrapie-infected sheep: determining the limit of sensitivity by immunohistochemistry. Clinical and Diagnostic Laboratory Immunolology. 2002. v. 9(2). p. 499-502.

Tuo, W, O'Rourke, K.I, Zhuang, D., Cheevers, W.P, Spraker, T.R, Knowles, D.P. Pregnancy status and fetal prion genetics determine PrPSc accumulation in placentomes of scrapie-infected sheep. Proceeding of the National Academy of Science USA. 2002. v. 99. p. 6310-6315.

Tuo, W, Zhuang, D, Knowles, D.P, Cheevers, W.P, Sy, M-S, O'Rourke, K.I. PrP-C and PrP-Sc at the maternal-fetal interface. Journal of Biological Chemistry. 2001. v. 276. p. 18229-34.

O'Rourke, K.I Ovine Scrapie: New tools for control of an old disease in veterinary clinics of North America. Food Animal Practice. 2001. v. 17(2) p. 283-300.

Herrmann, L.H, Davis, W.C, Knowles, D.P, Wardrop, K.J., Sy, M-S., Gambetti. P., O'Rourke, K.I. Cellular prion protein is expressed on PBMC but not platelets of normal and scrapie-infected sheep. Haematologica. 2001. v. 86. p. 164-171.

Kim, H, O'Rourke, K.I, Walter, M, Purchase, H.G, Enck, J, Shin, T.K. Immunohistochemical detection of scrapie prion proteins in clinically normal sheep in Pennsylvania. Journal of Veterinary Diagnostic Investigation. 2001. v. 13. p. 89-91.

Koo, H.C, Park, Y.H, Lee, B-C, Chae, C, O'Rourke, K.I, Baszler, T.V. Immunohistochemical detection of prion protein (PrP-Sc) and epidemiological study of BSE in Korea. Journal of Veterinary Science. 2001. v. 2(1). p. 25-31.

O'Rourke, K.I, Baszler, T.V, Besser, T.E, Miller, J.M, Cutlip, R.C, Wells, G.A.H, Ryder, S.J., Parish, S.M, Hamir, A.N, Cockett, N.E, Jenny, A. Knowles, D.P. Preclinical diagnosis of scrapie by immunohistochemistry of third eyelid lymphoid tissue. Journal of Clinical Micrology. 2000. v. 38. p. 3254-3259.

Herrmann, L.M, Baszler T.V, Knowles D.P. PrP(c) mRNA, but not PrP(Sc) is found in the salivary glands of scrapie-infected sheep. Biochimica et Biophysica Acta. 2000. v. 1479. p. 147-54.

Li, R., Liu, T., Tao, P., Morillas, M., Swietnicki, W., O'Rourke, K.I., Gambetti P., Surewicz, WK, Sy, M-S. Identification of an epitope in the C-terminus of normal prion protein whose expression is modulated by binding events in the N-terminus. Journal of Molecular Biolology. 2000. v. 301. p. 567-573.

Animal Diseases Research Unit

Project Title: Functional Approaches to the Control of Babesiosis in Cattle and Horses
CRIS Number: 5348-32000-020-00D
Scientists: Suarez, C., Knowles Jr, D., Scoles, G., Goff, W.
Location: Pullman, WA, Animal Diseases Research
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PROJECT OBJECTIVES:

There is a threat of reintroducing bovine babesiosis, a tick borne, hemoparasitic protozoal disease, into the U.S. from Mexico for the following reasons: 1) the USDA-APHIS surveillance program involves ticks only, 2) at least a million cattle are moved north across the Mexican border each year, a percentage of which are Babesia carriers, 3) acaracide resistant ticks occur in northern Mexico, 4) there is an increase in the number of wild ungulates along the border, and these and some cattle are not treated for ticks, and 5) there is no babesiacidal drug or vaccine approved for use in the U.S. Two products from research that would help alleviate the threat would be safe and effective vaccines for use in the U.S (and elsewhere) and diagnostic assays capable of handling large numbers of samples for use in surveillance. Babesia vaccine development requires the characterization of the protective immune mechanisms, the identification of protective antigens from the parasites, and the development of effective delivery systems. Given this, we have three areas of focus: 1) A series of immunologic assays have been developed and are now routinely used in our laboratory to characterize the immune response following exposure to Babesia and to assess the efficacy of immunization trials. These assays include, gene expression of several immunoregulatory cytokines, cytokine production and secretion assay by flow cytometry, lymphocyte, dendritic cell, and monocyte/macrophage activation, leukocyte phenotype analysis, cellular proliferation, and antibody isotype analysis. 2) In collaboration with scientists of the Department of Veterinary Microbiology and Pathology of WSU, we are characterizing new Babesia antigens as potential vaccine candidates, and investigating immunological properties of CpG containing DNA motifs present in the babesial genome. These CpG motifs have strong immunostimulatory activity. 3) We are establishing a babesial transfection system to functionally characterize genes expressing antigens of interest and to develop transmission-defective attenuated parasites. Reagents have also been identified for the design of an improved diagnostic assay for *Babesia bovis* and *B. bigemina*, and formatting and validating them are included in the objectives.

OVERALL PROJECT ACCOMPLISHMENTS:

Several assays used to evaluate the bovine immune response were developed and applied in studies providing a more complete understanding of the mechanisms operative during Babesia infection. One important mechanism involves the babesiacidal activity of nitric oxide and its induction by signals sent from immunologically activated cells. Among these signals are interferon-gamma and interleukin-12, both characteristic of a "Type-1 response". Other studies confirmed the importance of a Type-1 response in resolution of a primary Babesia infection, and suggested that the response is tightly regulated. Other molecules associated with a "Type-2 response", IL-4 and especially IL-10 have been shown to down-regulate the appropriate Type-1 response. Additional studies revealed the importance of Type-1 immunity against this

infection as a part of the strong innate immunity of young calves, and that the mechanisms are less effective in older cattle, where the disease is more severe. Natural killer (NK) cells capable of producing interferon gamma and that proliferate in the spleens of calves mounting a successful immune response have been identified. Reagents to purify both splenic dendritic cells and NK cells have recently been used to develop assays for examining the cooperative nature of these cells in response to babesial parasites *in vitro* as well as *ex vivo* during infection.

Work on the elucidation of the role of CpG motifs as an immunomodulator and the identification of new vaccine candidate antigens has and will further contribute to the design of future vaccines. A transition from the previous project design to a new project with more emphasis on the tick/pathogen interface was started in 2003. While studies concerning the immunology associated with babesial infection/immunization continue, the new project emphasizes the identification of babesial molecules involved in the transmission stages of the disease and the development of a novel babesial transfection system for genetic manipulation of the parasites. Recently, we developed a transient transfection system for expression of exogenous genes in Babesia bovis under the transcriptional control of the B. bovis rap-1 promoter. A B. bovis-specific protein that had been identified and characterized previously, was chosen for use as an epitope-defined diagnostic antigen, was formatted as a competitive ELISA assay and has resulted in good diagnostic specificity. Validation studies continue in efforts to establish the predictive value for the assay, and to determine conditions appropriate for commercialization. The work has resulted in the identification of parasite components with attributes important for the design of improved diagnostics (competitive ELISAs for each parasite (Babesia equi and Babesia caballi). Validation of these assays for adoption as replacements for the current official, but inferior assays has been accomplished. Improved diagnostics will improve and support the credibility of import regulatory policies. Basic immunology studies have revealed the importance of the disease agent-specific arm of the immune system involving T-cells in the resolution of infection whereas animals lacking T-cells suffered severe disease despite possessing the intact non-specific arm of the immune system.

IMPACT:
The tick-borne diseases laboratory within the ADRU has been invited to apply for OIE reference laboratory status acknowledging the development and standardization of diagnostic assays for equine and bovine babesiosis for use throughout the world. Our diagnostic tests for *Babesia equi* and *Babesia caballi* have been adapted by APHIS, are replacing the inaccurate CFT for importation testing and have been adopted by OIE as the prescribed tests for these diseases.
Basic immunology studies have characterized the protective innate immune response in cattle to *Babesia bovis* infection identifying critical aspects that need to be induced by new vaccines. The project has resulted in a battery of immunological assays with which to evaluate vaccine trials.

TECHNOLOGY TRANSFER:
Material transfer agreements: The cELISA for diagnosing *B. bovis* in cattle has been transferred to collaborators in Morocco, Australia, and Italy for validation. The improved diagnostic assays for equine babesiosis have been transferred to APHIS and to collaborators in Morocco in support of a FAS-supported epidemiology study. The equine babesiosis assays are being adopted for use by collaborators in Argentina, Brazil, Canada, England, Israel, Japan, and Morocco and as part of an OIE inter-laboratory validation.

Five improved tick-borne disease diagnostic assays have been transferred to the Institut Agronomique et Veterinaire (Hassan II) in Rabat, Morocco in support of ongoing epidemiology studies to determine the

feasibility of renewed trade activities with the US cattle industry. Moroccan graduate students were trained during the course of activities.
(Jehle, P., Goff, W.L. Scientific exchange for Moroccan technicians engaged in research on tick-borne disease control in North Africa. AgLink U.S.-Morocco Trade Promotion Program-1998-2003. 2003. End of Project Report. p. 49.)

Our diagnostic tests for equine babesiosis have been transferred to industry and will be commercially available within 2004. The company and APHIS are collaborating to assure standardization of test parameters and utilization.

PUBLICATIONS:

Suarez CE, Florin-Christensen M, Hines SA, Palmer GH, Brown WC, McElwain TF. Characterization of allelic variation in the Babesia bovis merozoite surface antigen 1 (MSA-1) locus and identification of a cross-reactive inhibition-sensitive MSA-1 epitope. 2000. Infection and Immunity. v. 68. p. 6865-6870.

Florin-Christensen J, Suarez CE, Florin-Christensen M, Hines SA, McElwain TF, Palmer GH. Phosphatidylcholine formation is the predominant lipid biosynthetic event in the hemoparasite Babesia bovis. 2000. Molecular and Biochemical Parasitology. v. 106. p. 147-156.

Brown WC, Ruef BJ, Norimine J, Kegerreis KA, Suarez CE, Conley PG, Stich RW, Carson KH, Rice-Ficht AC. A novel 20-kilodalton protein conserved in Babesia bovis and B. bigemina stimulates memory CD4(+) T lymphocyte responses in B. bovis-immune cattle. 2001. Molecular and Biochemical Parasitology. v. 118. p. 97-109.

Goff, W.L., Johnson, W.C., Parish, S.M., Barrington, G.M., Tuo, W. and Valdez, R.A. The age-related immunity in cattle to Babesia bovis infection involves the rapid induction of IL-12, IFN-□ and iNOS mRNA expression in the spleen. 2001. Parasite Immunology. v. 23. p. 453-461.

Shoda LK, Kegerreis KA, Suarez CE, Roditi I, Corral RS, Bertot GM, Norimine J, Brown WC. DNA from protozoan parasites Babesia bovis, Trypanosoma cruzi, and T. brucei is mitogenic for B lymphocytes and stimulates macrophage expression of interleukin-12, tumor necrosis factor alpha, and nitric oxide. 2001. Infection and Immunity. v. 69. p. 2162-2171.

Johnson, W.C., Lasri, S., Kappmeyer, L.S., Rhalem, A., Sahibi, H., Knowles, D.P. and Goff, W.L. Factors influencing the deployment of a Babesia caballi cELISA in tropical areas. 2001. Proceedings of the10th International Symposium of Veterinary Laboratory Diagnosticians, Parma, Italy. p. 322-323.

Rhalem, A., Sahibi, H., Lasri, S., Johnson, W.C., Kappmeyer, L.S., Hamidouch, A., Knowles, D.P. and Goff, W.L. Validation of a competitive enzyme-linked immunosorbent assay for diagnosing Babesia equi infections of Moroccan origin and its use in determining the seroprevalence of B. equi in Morocco. 2001. Journal of Veterinary Diagnostic Investigation. v. 13. p. 249-251.

Florin-Christensen M, Suarez CE, Hines SA, Palmer GH, Brown WC, McElwain TF. The Babesia bovis merozoite surface antigen 2 locus contains four tandemly arranged and expressed genes encoding immunologically distinct proteins. 2002. Infection and Immunity. v. 70. p. 3566-3575.

Norimine J, Suarez CE, McElwain TF, Florin-Christensen M, Brown WC. Immunodominant epitopes in Babesia bovis rhoptry-associated protein 1 that elicit memory CD4(+)-T-lymphocyte responses in B. bovis-immune individuals are located in the amino-terminal domain. 2002. Infection and Immunity. v. 70. p. 2039-2048.

Goff, W.L., Johnson, W.C., Parish, S.M., Barrington, G.M., Elsasser, T.H., Davis, W.C., and Valdez, R.A. IL-4 and IL-10 inhibition of IFN gamma- and TNF alpha-dependent nitric oxide production from bovine mononuclear phagocytes exposed to Babesia bovis merozoites. 2002. Veterinary Immunology and Immunopathology. v. 84. p. 237-251.

Stiller, D., Goff, W.L., Johnson, L.W. and Knowles, D.P. Dermacentor variabilis, Say (Acari: Ixodidae): an experimental vector of Babesia equi (Laveran) to ponies. 2002. Journal of Medical Entomology. v. 59. p. 667-670.

Goff, W.L., Johnson, W.C., Tuo, W., Valdez, R.A., Parish, S.M., Barrington, G.M. and Davis, W.C. The age-related innate immune response in calves to Babesia bovis involves IL-12 induction and IL-10 modulation. 2002. Annals of the New York Academy of Sciences. v. 969. p. 164-168.

Boonchit, S., Xuan, X., Yokoyama, N., Goff, W.L., Wagner, G.G. and Igarashi, I. Evaluation of an enzyme-linked immunosorbent assay with recombinant rhoptry-associated protein-1 antigen against Babesia bovis for the detection of specific antibodies in cattle. 2002. Journal of Clinical Microbiology. v. 40. p. 3771-3775.

Norimine J, Mosqueda J, Suarez C, Palmer GH, McElwain TF, Mbassa G, Brown WC. Stimulation of T-helper cell gamma interferon and immunoglobulin G responses specific for Babesia bovis rhoptry-associated protein 1 (RAP-1) or a RAP-1 protein lacking the carboxy-terminal repeat region is insufficient to provide protective immunity against virulent B. bovis challenge. 2003. Infection and Immunity. v. 7. p. 5021-5032.

Goff, W.L., McElwain, T.F., Suarez, C.E., Johnson, W.C., Brown, W.C., Norimine, J., and Knowles, D.P. Competitive-enzyme-linked immunosorbent assay based on a rhoptry-associated protein-1 epitope specifically identifies Babesia bovis infected cattle. 2003. Clinical and Diagnostic Laboratory Immunology. v. 10. p. 38-43.

Goff, W.L., Johnson, W.C., Horn, R.H., Barrington, G.M. and Knowles, D.P. The innate immune response in calves to Boophilus microplus tick transmitted Babesia bovis involves type-1 cytokine induction and NK-like cells in the spleen. 2003. Parasite Immunology. v. 25. p. 185-188.

Boonchit, S., Xuan, X., Yokoyama, N., Goff, W.L., Waghela, S.D., Wagner, G.G. and Igarashi, I. An improved enzyme-linked immunosorbent assay using C-terminal truncated recombinant antigens of Babesia bovis rhoptry-associated protein-1 for the detection of specific antibodies. 2004. Journal of Clinical Microbiology. v. 42. p. 1601-1604.

Goff, W.L., Knowles, D.P., Rhalem, A. and Sahibi, H. Equine piroplasmosis: a review with focus on improved diagnostics. Proceedings of the 1st Moroccan Conference of Equine Health and Reproduction. Rabat, Morocco (in press).

Shoda, L. K. M., Kegerreis, K. A., Suarez, C. E., Mwangi, W., Knowles, D. P., and Brown, W. C. 2001. Immunostimulatory CpG-modified plasmid DNA enhances IL-12, TNF-α, and NO production by bovine macrophages. Journal of Leukocyte Biology. 70:103-112.

Knowles, D. 2002. Equine Babesiosis, The five-minute veterinary consult: Brown, C. M., and Bertone, J. ED, Lippincott Willaims and Wilkins, Baltimore, MD, pp 160-161. (BOOK CHAPTER)

Ueti, M. W., Palmer, G. H., Kappmeyer, L. S., Scoles, G. A., and Knowles, D. P. 2003. Expression of equi merozoite antigen 2 during development of Babesia equi in the midgut and salivary gland of the vector tick *Boophilus microplus*. Journal of Clinical Microbiology. 41:5803-5809.

Knowles, D. P. 2003. Equine Babesiosis (Piroplasmosis) – A barrier in the international movement of horses. Equine Disease Quarterly 12:3-4. (Invited Review).

Animal Disease Research Unit

Project Title: BSE and Other TSEs Ruminant Herbivors: Diagnosis, Transmission and Genetics
CRIS Number: 5348-32000-021-00D
Scientists: O'Rourke, K.I., Knowles, D.P., Alverson, J.
Location: Pullman, WA, Animal Diseases Research Unit
Contact: O'Rourke, K. Phone: (509) 335-6020, Fax: (509) 335-8328, korourke@vetmed.wsu.edu

PROJECT OBJECTIVES:
Free ranging and farm raised deer and elk in the U.S. develop a fatal prion disorder, chronic wasting disease (CWD). CWD is a relatively uncharacterized TSE with novel patterns of transmission and agent distribution, as well as the considerable challenge of a persistent disease in free ranging wildlife. The objectives of the project are to develop inexpensive, high capacity live animal and postmortem diagnostic tests, prion detection methods for monitoring environmental contamination, and pathogenesis/transmission experiments that provide direction to intervention techniques and reagents. Control of all domestic prion diseases is important in reducing trade barriers for U.S. sheep and germplasm and for assuring the present and future global markets for cattle.

OVERALL PROJECT ACCOMPLISHMENTS:
Postmortem diagnostic test methodology for BSE using automated, monoclonal antibody immunohistochemistry was developed and transferred to USDA, APHIS, National Veterinary Services Laboratory. A non-automated immunohistochemistry test for BSE was developed in conjunction with private industry and is marketed outside the United States, with all sales registered with USDA, APHIS, Center for Veterinary Biologics. Postmortem diagnostic test methodology for CWD using automated immunohistochemistry was developed through a collaborative project with the Colorado State Veterinary Diagnostic Laboratory and transferred to USDA APHIS. The test is conducted by the National Veterinary Services Laboratory and a network of 20 contract laboratories and is used as a gold standard test to confirm the results on rapid (ELISA) tests. The test was modified for use in live deer using tonsil biopsies collected under general anesthesia. Monoclonal antibodies useful in assays on routinely formalin fixed tissue from infected sheep, deer, elk, cattle, humans, mink, domestic cats and a wide variety of captive wildlife potentially exposed to prion diseases were developed. Prion gene variation in mule deer, white-tailed deer, and elk was examined. A processed prion pseudogene, the first reported in placental mammals, was discovered to occur in a large proportion of mule deer and a small proportion of white-tailed deer. Genetic variants associated with relative susceptibility to CWD were reported in white-tailed deer and elk; no evidence of relative genetic resistance to CWD was found in mule deer.

IMPACT:
The development of an automated monoclonal antibody-based immunohistochemistry diagnostic assay has allowed the US to provide gold standard testing for BSE and CWD at NVSL and a network of 20 contract laboratories. The manual version of the test is the legal diagnostic test for BSE in Canada and in 17 countries in Central and South America. This standardized approach to diagnosis in the US and with our Western Hemisphere trading partners has allowed the cooperating nations to apply a single diagnostic standard for these diseases and increase the confidence of the regulatory agencies and US consumers in the validity of the

surveillance programs. The finding of a processed pseudogene in some species of deer has allowed the regulatory and research communities to correct previous work on genetic susceptibility and sets the stage for large scale field studies on transmission of the disease among related and unrelated members of free ranging breeding groups.

TECHNOLOGY TRANSFER:

Licenses:

VMRD, Inc., licensed the monoclonal antibody developed for this project and completed the research and development work on an inexpensive "dot blot ELISA" assay for early diagnosis of chronic wasting disease in mule deer and white tailed deer. VMRD, in conjunction with ADRU, developed an inexpensive immunohistochemistry diagnostic test for BSE. The test was validated by the Canadian Food Inspection Agency and the Veterinary Laboratories Agency in the United Kingdom. The BSE test is marketed internationally and is a diagnostic gold standard test in Canada. Monoclonal antibody F99/97.6.1 has been licensed by a number of companies attempting to develop antemortem tests and is available commercially for research purposes.

PUBLICATIONS:

O'Rourke, K.I, Spraker, T.R, Hamburg, L.K, Besser, T.E, Brayton, K.A, Knowles, D.P. Polymorphisms in the prion precursor functional gene but not the pseudogene are associated with susceptibility to chronic wasting disease in white-tailed deer. Journal of General Virology. 2004. v. 85. p. 1339-1346.

Spraker, T.R, Balachandran, A., Zhuang, D., O'Rourke, K.I. Variable patterns of PrP-CWD distribution in obex and cranial lymphoid tissues of Rocky Mountain elk with nonclinical chronic wasting disease. Veterinary Record . 2004 (in press).

Brayton, K.A, O'Rourke, K.I, Lyda, A.K, Miller, M.W, Knowles, D.P. A processed pseudogene contributes to apparent mule deer prion gene heterogeneity. Gene. 2004. v. 326. p. 167-173.

O'Rourke, K.I., Zhuang, D., Lyda, A., Gomez, G., Williams, E.S., Tuo, W., Miller, M.W. Abundant PrP-CWD in tonsil from mule deer with preclinical chronic wasting disease. Journal of Veterinary Diagnostic Investigation 2003. v. 15. p. 320-323.

Spraker, T.R., Zink, R.R., Cummings, B.A., Sigurdson, C.J., Miller, M.W., O'Rourke, K.I. Distribution of protease-resistant prion protein and spongiform encephalopathy in free-ranging mule deer (Odocoileus hemionus) with chronic wasting disease. Veterinary Pathology. 2002. v. 39. p. 546-556.

Wild, M.A., Spraker, T.R., Sigurdson, C.J., O'Rourke, K.I., Miller, M.W. Preclinical diagnosis of chronic wasting disease in captive mule deer (Odocoileus hemionus) and white-tailed deer (Odocoileus virginianus) using tonsillar biopsy. Journal of General Virology. 2002. v. 83. p. 2629-2634.

Spraker, T.R, O'Rourke, K.I, Balachandran, A., Zink, R.R, Cummings, B.A, Miller, M.W., Powers, B.E. Validation of monoclonal antibody F99/97.6.1 for immunohistochemical staining of brain and tonsil in mule deer (Odocoileus hemionus) with chronic wasting disease. Journal of Veterinary Diagnostic Investigation. 2002. v. 14. p. 3-7.

Spraker, T.R, Zink, R.R, Cummings, B.A, Wild, M.A, Miller, M.W, O'Rourke, K.I. Comparison of histological lesions and immunohistochemical staining of proteinase resistant prion protein in a naturally-occurring spongiform encephalopathy of free-ranging mule deer (Odocoileus hemionus) with those of chronic wasting disease of captive mule deer. Veterinary Pathology. 2002. v. 39. p. 110-119.

Raymond, G.J, Bossers, A., Raymond, L.D, O'Rourke, K.I, McHolland, L.E, Bryant, P.K, Miller, M.W, Williams, E.S, Smits, M., Caughey, B. Evidence of a molecular barrier limiting susceptibility of humans, cattle and sheep to chronic wasting disease. EMBO Journal. 2000. v. 19. p. 4425-4430.

Hamir, A.N, Cutlip, R.C, Miller, J.M, Williams, E.S, Stack, M.J, Miller, M.W, O'Rourke, K.I, Chaplin, M.J. Preliminary findings on the experimental transmission of chronic wasting disease agent of mule deer to cattle. Journal of Veterinary Diagnostic Investigation. 2001. v. 13. p. 91-96.

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PROJECTS

Southeast Poultry Research Laboratory (SPRL)

- Poult Enteritis Mortality Syndrome
- Identification of Virulence Determinants, Pathogenetic Mechanisms and Control Methods of Avian Paramyxoviruses
- Application of Molecular Biological Techniques to the Diagnosis and Control of Avian Influenza
- Mucosal immunization to protect poultry against avian influenza.
- Development and Validation of Rapid Diagnostic Tests for Avian Influenza and Newcastle Disease
- Development of environmental and genomic strategies to control Marek's disease

Southeast Poultry Research Laboratory

CRIS Title: Poult Enteritis Mortality Syndrome
CRIS: 6612-32000-036-00D
Scientists: Spackman, E.; Pantin-Jackwood, M.
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PROJECT OBJECTIVES:
Poult enteritis mortality syndrome (PEMS) is a highly infectious disease of young turkeys. PEMS was first reported in North Carolina in 1991. Since then, PEMS has been reported throughout the Southeastern United States and Texas. There are also reports of a PEMS-like disease in California, Arkansas, Missouri, and Israel. PEMS has been called one of the most devastating emergent diseases to strike the poultry industry due to production losses from the disease complex and in some regions of the U.S. an estimated 60-90% of all commercial turkey flocks will experience some poult enteritis like disease seasonally during grow-out. The causative agent(s) of PEMS remains unknown. The research under this CRIS seeks to identify the PEMS agent(s) using novel molecular techniques, develop diagnostic reagents for rapid identification, and understand the pathogenesis of the disease.

OVERALL PROJECT ACCOMPLISHMENTS:
Accomplishments over the life of this project have included: 1) the isolation, identification and characterization of a unique strain of astrovirus in turkeys with PEMS; 2) reproduction of a PEMS-like clinical disease in turkeys with this astrovirus; 3) reproduction of immune deficiencies in turkeys with the astrovirus which reduces the ability of turkeys to fight off other pathogens; 4) an evaluation of commercial disinfectants which demonstrated that most fail to inactivate the astrovirus, with the exception of formaldehyde and Virkon S that were effective for killing the virus; 5) the development of a specific and sensitive standard RT-PCR test to detect turkey astrovirus and transfer of the technology to veterinary diagnostic laboratories; 6) development of state-of-the-art molecular tests (multiplex real-time-RT-PCR) for the simultaneous detection of several PEMS associated viruses, including; turkey astrovirus, turkey coronavirus, classical avian reoviruses and turkey reoviruses, all from samples collected from PEMS affected turkeys; 7) an evaluation of turkey astrovirus, turkey coronavirus and turkey reovirus shed and virus presence in selected organs at different times post-exposure in order to determine optimal sampling methods for the detection of each virus with molecular tests; and 8) a preliminary characterization of turkey reoviruses which are associated with poult enteritis complex.

IMPACT:
This work has directly addressed the objective of developing rapid, sensitive and accurate detection methods for the viruses commonly associated with PEMS (turkey coronavirus, turkey astrovirus and avian reovirus). Also optimal sampling methods have been determined to increase the utility of these methods in the field. The availability of accurate molecular diagnostic tests using state-of-the-art technology, such as real-time RT-PCR will also help to address the objective of isolating, identifying and characterizing viral agents associated with PEMS due to the difficulty in culturing these agents. Additionally, using the improved technology we have been able to identify reoviruses that are genetically different from classical avian reoviruses in samples collected from commercial turkey flocks with poult enteritis and which otherwise may not have been detected.

Therefore more pathogens can be detected and more information about them can be obtained at their initial detection.

TECHNOLOGY TRANSFER:
Schultz-Cherry, S., L.A. Kelley, M.D. Koci and B.S. Seal. Avian Astrovirus US patent# 6,696,562

A reverse-transcription-polymerase chain reaction (RT-PCR) was developed, based on the sequence produced at SEPRL, to identify the turkey astrovirus, and the test was transferred to the University of Guelph diagnostic laboratory. The RT-PCR test was further enhanced into a multiplex format so that could identify both turkey astroviruses and turkey coronaviruses from the same sample. This test was transferred for use by the Poultry Diagnostic Research Center at the University of Georgia.

PUBLICATIONS:

Koci, M.D., Seal, B.S., Schultz-Cherry, S. Development of an RT-PCR diagnostic test for an avian astrovirus. 2000. Journal of Virological Methods. v.90 pp. 79-83

Schultz-Cherry, S., Kapczynski, D.R. Simmons, V.M. Koci, M.D., Brown, C., Barnes, H.J. Identifying Agent(s) associated with poult enteritis mortality syndrome; importance of the thymus. 2000. Avian Diseases. v.44. pp. 256-65.

Schultz-Cherry, S., Kind, D.J., Koci, M.D. Inactivation of an astrovirus associated with poult enteritis mortality syndrome. 2001. Avian Diseases. v.45. pp. 76-82.

Kapczynski, D.R., Sellers, H.S., Simmons, V., Schultz-Cherry, S. Sequence analysis of the S3 gene from a turkey reovirus**.** Virus Genes. 2002. v.25. p.95-100.

Koci M.D., Kelley, L.A., Larsen, D. and S. Schult-Cherry. Astrovirus induced synthesis of nitric oxide contributes to virus control during infection, Journal of Virology, 78:1564-74, 2004

Sellers, H.S., M.D. Koci, E. Linneman, L.A.Kelley and S. Schultz-Cherry. Development of a multiplex reverse transcription-polymerase chain reaction diagnostic test specific for turkey astrovirus and coronavirus. Avian Diseases; in Press

Southeast Poultry Research Laboratory

CRIS Title: Identification of Virulence Determinants, Pathogenetic Mechanisms and Control Methods of Avian Paramyxoviruses
CRIS: 6612-32000-038-00D
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PROJECT OBJECTIVES:
Avian paramyxoviruses are the cause of two diseases important to the U. S. poultry industry. Newcastle disease (ND) is a viral disease of poultry and a worldwide problem first recognized in the U. S. in the 1930s. Newcastle disease virus (NDV), also known as avian paramyxovirus type 1 (APMV-1), infects all known wild and domestic bird species. Different NDV strains vary in virulence and produce differing clinical forms of the disease that range from high mortality to mild or inapparent respiratory infections. Although the highly virulent form of the disease was diagnosed in the U.S. in 2002-2003 for the first time since 1998, the mild form of ND is the predominant one occurring in the U.S. and is the cause of reduced productivity from respiratory disease and reduced rate of gain. Avian pneumovirus (APV) infections of turkeys in the U.S., in contrast to ND, are of recent concern. APV, now classified as an avian metapneumovirus, was isolated from commercial turkeys in Colorado in 1996. The disease was reported in the United Kingdom in 1985 and prior to 1996 the disease was exotic to North America. The virus causes a mild, but rapidly spreading, upper respiratory disease and adverse effect on weight gain and feed conversion. Secondary bacterial infections increase the severity of the disease. The initial diagnosis of the disease in the U.S. was delayed because the U.S. isolate was of a different subtype than had been isolated elsewhere and serological assays to detect the new subtype had to be developed. Research to resolve the problems caused by these two diseases includes characterization of new isolates, identifying the factors responsible for their causing disease, and developing control strategies, including vaccination and diagnostics.

OVERALL PROJECT ACCOMPLISHMENTS:
Present methods for the detection of the causative agent of Newcastle disease (ND), Newcastle disease virus (NDV), are based on isolation of the virus in embryonated chicken eggs, a sensitive and reliable method but expensive and time consuming. ND susceptible and vaccinated chickens and turkeys were infected with the California 2002 END virus at Southeast Poultry Research Laboratory (SEPRL) to provide samples for comparative testing by virus isolation and a rapid diagnostic method developed by SEPRL scientists from the avian influenza and Newcastle disease rapid diagnostic development project. A comparison of results of virus isolation and the rapid test demonstrated the efficacy of the newly developed rapid test procedure. This efficacy with experimental samples was the justification for proceeding with validation testing of the new rapid procedure on field samples including those from the END outbreak of 2002-2003 and transfer of the test to the National Veterinary Services Laboratories for use in the California NDV outbreak.

Last year, avian pneumovirus (APV) antibody was demonstrated in wild birds in the Southeast U.S. and suggests wild birds could be a source of APV virus transmission to turkeys. Southeast Poultry Research Laboratory scientists collected swabs from the species of wild birds previously found to have APV antibodies. APV was identified from 12 of the sampled wild birds and it was determined through sequence analysis of the

glycoprotein, matrix and fusion genes that the wild bird viruses are closely related to viruses found in domestic poultry. The presence of virus in wild birds and the relatedness to viruses found in domestic poultry indicate the potential for wild birds to spread APV infections in areas of the United States where the virus is currently not found in poultry.

Identifying the source of the exotic Newcastle disease (END) virus that caused the END outbreak in the Southwestern U.S. and Texas during 2002-2003 is critical to the development of control programs to prevent future outbreaks. Southeast Poultry Research Laboratory scientists analyzed and compared the nucleotide sequence of the virus genome of isolates from the 2002-2003 outbreaks with the sequence of recent isolates of END virus from U.S. quarantine stations and from outbreaks in several countries in the world. The END virus isolates from the 2002-2003 U.S. outbreaks were found to be most similar to viruses isolated from northern Mexico during 1996 and 2000. Therefore, the most likely source of END virus that caused the California and Texas 2002-2003 END outbreaks was virus transmission from Mexico via infected birds or virus contaminated fomites.

Genetic changes in avian pneumovirus (APV) between its initial identification as the cause of turkey rhinotracheitis (TRT) in Colorado during 1997 and the isolates from current outbreaks in Minnesota were unknown. Southeast Poultry Research Laboratory scientists analyzed the nucleotide sequence of the virus attachment protein gene (G) of isolates from 1997 and compared it to the G sequence of recent APV isolates. APV virus G gene changes were found to have occurred between 1997 and the present. These changes suggest selection or mutation among U.S. isolates has occurred since the first outbreak of TRT in the U.S.

The embryonated chicken egg had not been examined as a model system to evaluate virulence properties of Newcastle disease virus (NDV) isolates and laboratory induced NDV mutations. At the Southeast Poultry Research Laboratory, the embryos from eggs inoculated with NDV reference and mutated reference strains were collected, formalin fixed, sectioned, and stained with a gene probe and NDV antibodies to detect virus distribution in the tissues and membranes of the embryonated egg. Low-virulence strains were detected exclusively in the cells of the chorioallantoic membrane, whereas more virulent NDV isolates and mutated strains that had been demonstrated to have acquired virulence for chickens were widely disseminated in chicken embryo tissues. The results demonstrate the potential value of this as a model system for evaluating NDV virus virulence as an alternative to chicken inoculation.

Efficacious avian pneumovirus (APV) vaccines are needed by the turkey industry. At the Southeast Poultry Research Laboratory, a live attenuated APV vaccine (cp30) was developed by successive adaptation of the virus to decreasing temperatures and its efficacy in turkeys was evaluated. The results indicate the dose necessary for complete protection from clinical signs following APV challenge also resulted in clinical signs following vaccination. A cold adaptation of the vaccine strain to reduce post vaccinal reaction was ineffective at reducing those reactions at a dosage required to produce protection.

Protection of commercial poultry from exotic Newcastle disease (END) is a constant threat to U.S. poultry producers. Southeast Poultry Research Laboratory scientists conducted vaccine efficacy experiments with END California 2002 virus in chickens. The results indicated protection from END with either a single dose of live or inactivated vaccine. Current commercial ND vaccines are effective in protecting against disease resulting from END virus infections.

The capability to easily differentiate vaccinated from infected birds would enhance current Newcastle disease (ND) control programs. A project scientist developed a vaccine that is a shell of the whole virus which retains the important surface structures that induce immunity but can't infect or be transmitted from bird to bird because the virus genome and internal proteins have been removed. The vaccine is known as a Newcastle disease virus (NDV) virosome vaccine, was administered intranasally, and protected chickens against a lethal NDV challenge. Because vaccinated birds can be differentiated from infected birds, this vaccine may provide an alternative to current live NDV vaccines.

Extensive genomic data of Newcastle disease virus (NDV) is largely incomplete. To extend that data, the nucleocapsid (N) protein genes from twenty-four NDV isolates representing various pathotypes with different geographical and chronological origins were cloned and sequenced. Comparison of N protein amino acid sequences among members of the Paramyxoviridae resulted in NDV and avian paramyxovirus type 6 separating as a cluster distinct from the Rubulavirus genus. This data provided further support for the recently updated classification of avian paramyxoviruses in their own genus Avulavirus among the Paramyxovirinae.

Rapid diagnostic procedures are needed for Newcastle disease virus (NDV). Viral genomic information around a known virulence marker of NDV was used to develop a heteroduplex mobility assay (HMA) to differentiate strains that have different genome sequences in the region of that marker. Project scientists were able to distinguish vaccine-like viruses from other isolates potentially virulent for chickens. Use of the HMA in labs that do not have nucleotide sequencing facilities can provide a rapid identification of potentially virulent NDV that continue to threaten commercial poultry.

Waterfowl have been a suspect source of avian pneumovirus (APV) that infected turkeys in Minnesota. University of Minnesota scientists placed APV-negative mallard ducks as sentinels on a turkey farm experiencing a severe APV outbreak and were able to recover APV from swab samples and detect anti-APV antibodies in serum of the sentinel ducks even though they did not develop clinical APV disease. Sequence analysis at Southeast Poultry Research Laboratory of four APV isolates recovered from the APV outbreak in turkeys shared 95% to 99% nucleotide sequence identity and 97% to 99% predicted amino acid sequence identity with the duck isolate. These results indicate that the APV isolates from turkeys and ducks shared a common source, and the viruses from different avian species can cross-infect.

The diversity of among avian pneumovirus (APV) isolates from commercial turkeys in the north-central region of the U.S., particularly Minnesota had not been determined. A comparison of nucleotide and predicted amino acid sequences from 15 U.S. AMPV isolates were completed. The results demonstrated that these viruses were all closely related and distinct from European APV isolates. This further confirms that the U.S. viruses are closely related genetically and form a separate sub-type relative to European viruses.

An effective APV vaccine is still needed to control the disease in turkeys. Turkeys were vaccinated with a DNA vaccine comprised of the APV fusion protein gene, a gene whose product is believed critical in generating an effective immune response. Vaccinated turkeys shed less challenge virus and produced higher neutralizing antibody titers than attained with other experimental vaccines. The results suggest that improved APV vaccines are feasible and provide the basis for expanding this vaccination approach.

Laboratory experiments with APV fail to reproduce the severity of disease seen in the field. Southeast Poultry Research Laboratory (SEPRL) scientists conducted coinfection experiments with APV and low virulent

Newcastle disease virus (NDV) or *Escherichia coli* at SEPRL. Coinfection of APV and NDV results in increased severity of disease. This suggests that control of field NDV or more judicious use of NDV vaccines are necessary to control APV.

Existing avian pneumovirus (APV) diagnostic tests do not work in bird species other than chickens and turkeys. A competitive ELISA test developed in FY2001 at Southeast Poultry Research Laboratory was validated using field sera samples in conjunction with Minnesota Bureau of Animal Health. Using the validated test, APV positive sera samples were identified in American coots and Canada geese but not in 13 other wild bird species. This suggests that some wild birds can be infected with APV and may be a reservoir for the virus.

IMPACT:
The work addresses the objectives in our OSQR Project Plan: 1) to characterize emergent Newcastle disease virus and avian metapneumovirus isolates to extend the capabilities for molecular epidemiology of the most important avian paramyxovirus infections; 2) to further characterize determinants important to avian paramyxovirus pathogenesis; and 3) to develop improved control strategies, including vaccination, diagnostics and identification of virus reservoirs based on information from avian paramyxovirus characterization and pathogenesis studies. The outbreak of exotic Newcastle disease (END) that was diagnosed in the U.S. during October 2002 served to focus and provide immediate impact for this year's research. Project scientists provided support in the eradication of END by analysis and experimentation that are components of the first and third project objectives.

TECHNOLOGY TRANSFER:
Patents:
"Nucleotide Sequence for the Avian Metapneumovirus (Colorado) Attachment Glycoprotein Gene" Inventors: Bruce S. Seal and Rene Alvarez. Docket No.: 0036.02. Serial No.: 10/285,626. Date Filed: 11/01/2002.

An invention report, No. 0186.02 entitled "A Virosome Vaccine for NDV", was submitted to the Office of Technology Transfer for potential patent development of the NDV virosome vaccine developed in our laboratory.

Cooperative Research and Development Agreements (CRADA):
CRADA #58-3K95-2-914 entitled "Improved Diagnostics for Avian Pneumovirus by Use of Peptide Antigens and Anti-peptide Antiserum to the Viral Nucleocapsid Protein and Phosphoprotein" between ARS and IDEXX Laboratories, Inc. Principal Investigator: Bruce Seal

PUBLICATIONS:

Seal, B. S., D. J. King, and R. J. Meinersmann. Molecular evolution of the Newcastle disease virus matrix protein gene and phylogenetic relationships among the paramyxoviridae. 2000. Virus Research. V. 66. P.1-11.

Seal, B. S., D. J. King, and H. S. Sellers. The avian response to Newcastle disease virus. 2000. Developmental and Comparative Immunology. V. 24. P. 257-268.

Seal, B.S., Sellers, H.S. and Meinersmann, R.J. Fusion protein predicted amino acid sequence of the first U.S. avian pneumovirus isolate and lack of heterogeneity among other U.S. isolates. 2000. Virus Research. V66. p.139-147

Locke, D.P., Sellers, H.S., Crawford, J.M., Schultz-Cherry, S., King, D.J., Meinersmann, R.J., Seal, B.S. Newcastle disease virus phosphoprotein gene analysis and transcriptional editing in avian cells. Virus Research. 2000. v. 69. p. 55-68.

Gulati, B.R., Cameron, K.T., Seal, B.S., Goyal, S.M., Halvorson, D.A., Njenga, M.K. Development of highly sensitive and specific enzyme-linked immunosorbent assay based on recombinant matrix protein for detection of avian pneumovirus antibodies. Journal of Clinical Microbiology. 2000. v.38. p. 4010-4014.

King, D.J. Selection of Thermostable Newcastle Disease Virus from Reference and Vaccine Strains. Avian Diseases. 2001. v. 45. p. 512-516.

Berinstein, A., Sellers, H.S., King, D.J., Seal, B.S. Use of a heteroduplex mobility assay to detect differences in the fusion protein cleavage site coding sequence among Newcastle disease virus isolates. 2001. Journal of Clinical Microbiology. v. 39. p. 3171-3178.

Kommers, G.D., King, D.J., Seal, B.S., Brown, C.C. Virulence of pigeon-origin Newcastle disease virus isolates for domestic chickens. 2001. Avian Diseases. v. 45. p. 906-921.

Sellers, H.S., Villegas, P., El-Attrache, J., Kapczynski, D.R., Brown, C.C. Detection of infectious bursal disease virus in experimentally infected chickens using in situ hybridization. Avian Diseases. 2001. v. 45. p. 26-33.

Kommers, G.D., King, D.J., Seal, B.S., Carmichael, K.P., Brown, C.C. Pathogenesis of six pigeon-origin isolates of Newcastle disease virus for domestic chickens. Veterinary Pathology. 2002. v. 39. p. 353-362.

Seal, B.S., Crawford, J.M., Locke, D.P., Sellers, H.S., King, D.J. Nucleotide sequence analysis of the Newcastle disease virus nucleocapsid gene and phylogenetic relationships among the Paramyxoviridae. Virus Research. 2002. v. 83. p. 119-129.

Shin, H.J., Nagaraja, K.V., McComb, B., Halvorson, D.A., Jirjis, F.F., Shaw, D.P., Seal, B.S, Njenga MK. Isolation of avian pneumovirus from mallard ducks that is genetically similar to viruses isolated from neighboring commercial turkeys. Virus Research. 2002. v. 83. p. 207-212.

Shin, H.-J., Cameron, K.T., Jacobs, J.A., Turpin, E.A., Halvorson, D.A., Goyal, S.M., Nagaraja, K.V., Kumar, M.C., Lauer, D.C., Seal, B.S., Njenga, M.K. Molecular epidemiology of subgroup C avian pneumoviruses isolated from the United States and comparison with subgroup A and B viruses. Journal of Clinical Microbiology. 2002. v. 40. p. 1687-1693.

Turpin, E.A., Perkins, L.E.L., Swayne, D.E. Experimental infection of turkeys with avian pneumovirus and either Newcastle disease virus or *Escherichia coli*. Avian Diseases. 2002. v.46. p.412-422.

Lwamba, H.C., Halvorson, D.A., Nagaraja, K.V., Turpin, E.A., Swayne, D., Seal, B.S., Njenga, M.K. Antigenic cross-reactivity among avian pneumoviruses of subgroups A, B, and C at the matrix but not nucleocapsid proteins. 2002. Avian Diseases. v. 46. p. 725-729.

Turpin, E.A., Perkins, L.E.L., Swayne, D.E. Experimental Infection of Turkeys with Avian Pneumovirus and Either Newcastle Disease Virus or *Escherichia coli*. Avian Diseases. 2002. v. 46. p. 412-422.

Alvarez, R., Lwamba, H.M., Kapczynski, D.R., Njenga, M.K., Seal, B.S. Nucleotide and predicted amino acid sequence-based analysis of the avian metapneumovirus type C cell attachment glycoprotein (G) gene: Phylogenetic analysis and molecular epidemiology of U.S. pneumoviruses. 2003. Journal of Clinical Microbiology. v. 41. p. 1730-1735.

Jacobs, J.A., Njenga, M.K. Alvarez, R., Mawditt, K., Britton, P., Cavanagh, D., Seal, B.S. Subtype B avian metapneumovirus resembles subtype A more closely than subtype C or human metapneumovirus with respect to the phosphoprotein, second matrix and small hydrophobic proteins. Virus Research. 2003. v. 92. p. 171-178.

Kommers, G.D., King, D.J., Seal, B.S., Brown, C.C. Pathogenesis of chicken-passaged Newcastle disease viruses isolated from chickens and wild, and exotic birds. 2003. Avian Diseases. v. 47. p. 319-329.

Kommers, G.D., King, D.J., Seal, B.S., Brown, C.C. Virulence of six heterogenous-origin Newcastle disease virus isolates before and after sequential passage in domestic chickens. 2003. Avian Pathology. v. 32. p. 81-93.

Njenga, M.K., Lwumba, H.M., Seal, B.S. Metapneumovirses in birds and humans. 2003. Virus Research. v. 92. p. 163-169.

Swayne, D.E., King, D.J.. Zoonosis update: avian influenza and Newcastle disease. 2003. Journal of the American Veterinary Medical Association. v. 222. p. 1534-1540.

Zanetti, F., Rodriquez, M., King, D.J., Capua, I., Carillo, E., Seal, B.S. Berinstein, A. Matrix protein gene sequence analysis of avian paramyxovirus 1 isolates obtained from pigeons. 2003. Virus Genes. v. 26. p. 199-206.

Kapczynski, D. R., Tumpey, T. M. Development of a virosome vaccine for Newcastle disease virus. 2003. Avian Dis. v. 47. p. 578-587.

Kapczynski, D. R., Sellers, H. S. Immunization of turkeys with a DNA vaccine expressing either the F or N gene of avian metapneumovirus. 2003. Avian Dis. v. 47. p. 1376-1383.

Turpin, E.A., Lauer, D.C. and Swayne, D.E. Development and evaluation of a blocking ELISA for the detection of type C avian metapneumovirus antibodies in multiple domestic avian species. Journal of Clinical Microbiology. 2003. v.41. p.3579-3583.

Donoghue, K., Lomniczi, B., McFerran, B., Conner, T.J., Seal, B., King, D., Banks, J., Manvell, R., White, P.S., Richmond, K., Jackson, P., Hugh-Jones, M.: Retrospective characterization of Newcastle disease virus Antrim '73 in relation to other epidemics, past and present. Epidemiol. Infect. 2004. v. 132. p. 357-368.

Wise, M.G., Suarez, D.L., Seal, B.S., Pedersen, J.C., Senne, D.A., King, D.J., Kapczynski, D.R., Spackman, E.: Development of a real-time, reverse-transcriptase PCR for detection of Newcastle disease virus RNA in clinical samples. J. Clin. Micro. 2004. v. 42. p. 329-338.

Alvarez, R., Njenga, M.K., Scott, M., Seal, B.S.: Development of a nucleoprotein-based enzyme-linked immunosorbent assay utilizing a synthetic peptide antigen for detection of avian metapneumovirus antibodies in turkey sera. Clin. Diagn. Lab. Immunol. 2004. v. 11. p. 245-249.

Southeast Poultry Research Laboratory

Project Title:	Application of Molecular Biological Techniques to the Diagnosis and Control of Avian Influenza
CRIS Number::	6612-32000-039-00D
Scientists:	Suarez, D., Mitchell, B., Spackman, E., Swayne, D., Vacant, vacant
Location:	Athens, Georgia Southeast Poultry Research Laboratory
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PROJECT OBJECTIVES:

Avian influenza presents a major disease threat to the US poultry industry. The unpredictable nature of the disease and the viral agent that causes it is the same today as it was in 1983 when the disease struck the Northeastern United States with severe economic consequences. Because of research on avian influenza viruses in recent years we now know that some viruses can rapidly change from causing only mild disease to one that causes a deadly disease in chickens. It is likely that the longer a virus infects commercial poultry, the more likely it is to cause the severe form of the disease. The research under this CRIS unit seeks to understand the changes that are required for this shift in ability to cause disease. The research also seeks to control the presence of AI viruses in poultry by development of new and more effective vaccines and to develop tests to more rapidly diagnosis infection in chickens.

The CRIS project proposal has four major objectives. They include:

1) Define the role of individual avian influenza virus (AIV) genes and the host responses in the pathogenesis and the host range of AIV.
2) Conduct research in molecular epidemiology of AIV outbreaks in poultry and wild birds to identify sources and reservoirs of AIV.
3) Develop and evaluate immunologic and chemotherapeutic control methods for AIV.
4) Develop rapid diagnostic tests for AIV.

The projects objectives are designed to flexible to respond to disease outbreaks, including outbreaks that affect poultry caused by other disease agents.

OVERALL PROJECT ACCOMPLISHMENTS:

In collaboration with APHIS (National Veterinary Services Laboratory, Ames, IA), a real time RT-PCR (RRT-PCR) test was developed that can rapidly and with high sensitivity detect both low pathogenic and highly pathogenic avian influenza. This test was validated for the direct detection of any influenza viruses from infected birds in an outbreak situation, and was used to help eradicate an H7 avian influenza outbreak in Virginia in 2002. The test was adopted as an official test by APHIS and is being used for surveillance throughout the United States, including by the National Animal Health Laboratory Network, the federally supported state diagnostic laboratory system. The test has continued to play a valuable role in initially diagnosing and helping to control avian influenza outbreaks in several different states, including Delaware,

Maryland and Texas. This was the first widespread use of a real-time RT-PCR for the control of a animal disease outbreak in the United States.

For the control of low pathogenic avian influenza outbreaks, vaccination is being more commonly considered, because it can potentially help control an outbreak at a lower cost than depopulation programs. At the Southeast Poultry Research Laboratory (SEPRL), the use of currently available and new vaccination strategies are being investigated for the control of avian influenza. Currently only two types of vaccines are available for use for avian influenza, killed adjuvanted vaccines and fowlpox-vectored vaccines. Our research has shown that to get optimal protection from these vaccines, it is important to match the vaccine to the challenge strain. A better match of vaccines allows less virus to be shed from vaccinated but infected birds. Additional research has shown that when vaccination is used on a widespread basis antigenic drift, similar to what is seen with human influenza viruses, can be a problem for decreased effectiveness for the vaccine. Our laboratory was the first to show that antigenic drift can be a problem for vaccination use for avian influenza viruses. Additional research has been focused on using viral-vectored or recombinant vaccines for avian influenza including fowlpox vectored vaccines, replication incompetent alphavirus vectors, and Newcastle disease virus vectored vaccines. All three of these vaccines types have shown to provide protection from influenza challenge, and can provide the advantage of use as a DIVA (differentiate infected from vaccinated animals) vaccine. These vaccines are still being evaluated to determine if they have significant advantages over commercially available vaccines and can be produced in a cost effective manner. Additional vaccine technologies, including the reverse genetics approach to create avian influenza viruses that can also be used with the DIVA approach have also been shown to be effective.

The characterization of influenza isolates in the U.S. and around the world is important to assess potential biological threats to the U.S. poultry industry. In collaboration with researchers at the National Veterinary Services Laboratory, state veterinary diagnostic laboratories, the Hong Kong Agriculture, Fisheries, and Conservation Department, the National Veterinary Research and Quarantine Service in South Korea, the Chile Ministry of Agriculture, and other countries, we have characterized influenza viruses from the U.S. and around the world by sequence analysis and animal inoculation studies. The molecular epidemiology and animal studies derived from this work is important for estimating the risk for each influenza outbreak and has resulted in the characterization of a novel way for a low pathogenic avian influenza to become highly pathogenic (Chile H7N3 outbreak). More recently, our characterization of H5N1 viruses from Southeast Asia has been valuable in demonstrating differences between viruses from other countries, and helping to identify risk factors for this country. Vaccination studies were also conducted that showed that current vaccines could still protect from birds from clinical disease for these viruses.

In the past several years, several countries including Mexico and Japan, have tried to block imports of U.S. poultry product based on the concern for virus being transmitted in poultry meat or egg products. Research done at SEPRL has shown for low pathogenic avian influenza, virus infection of meat did not occur and the risk of spread through meat products was low. Additionally, work done at SEPRL showed that commonly used pasteurization or processing

temperatures would inactivate virus in egg products, which makes these products safe even if they were exposed to avian influenza viruses. This information was shared directly with trade officials as part of APHIS trade missions, and provided some relief from unfounded trade restrictions.

Our laboratory has been partnering with several other laboratories in the U.S. and abroad to do wild bird surveillance for avian influenza. The goal of this work is to molecularly and antigenically characterize viruses from throughout the world to better understand the variability of influenza viruses in the natural host population. This information will prove valuable for preparing for disease outbreaks in the United States.

The Southeast Poultry Research Laboratory has an interest in emerging diseases that might impact poultry, and with their Biosafety-Level 3 facilities, are in a unique position to study some of these emerging diseases. Our laboratory was the first or one of the first laboratories to do poultry related research for both the Severe Acute Respiratory Syndrome (SARS) virus and West Nile virus in the U.S. The SARS virus emerged in Southeast Asia and infected over 8400 humans with over 800 deaths. Early epidemiologic evidence suggested a zoonotic potential for the virus with animals in the live animal markets in China. The source of the virus causing SARS is still unknown but animals may be the source or may be contributing to the spread of the virus. The Southeast Poultry Research Laboratory conducted a study to determine if chickens, turkeys, ducks, geese and Japanese quail were susceptible to the SARS virus and could spread the virus to humans. The study failed to demonstrate that the SARS virus could grow in these birds. These data indicate that the common five domestic poultry species were not the reservoir and will not spread the SARS virus to humans. Similar research with West Nile Virus was conducted that showed the common poultry species could be infected with these viruses, but clinical disease seldom occurred and that with low levels of viremia, these species were unlikely to spread the virus.

IMPACT:
Progress was made in all four major objectives of the CRIS project, and this work continues to have direct and indirect impact for the poultry industry. The development of a real-time RT-PCR for avian influenza provided an immediate benefit in helping to control the outbreak of avian influenza in Virginia. This new test not only provided results much faster than conventional virus isolation, it was cheaper and more tests could be performed each day. This test has become the official standard for surveillance by APHIS and is being used by over 20 diagnostic labs in the U.S. and abroad. The ongoing commitment to do molecular epidemiology for outbreaks in the U.S. and abroad has helped to provide the poultry industry information about what viruses pose risks to the U.S. poultry industry. Through the isolation and sequence analysis with both commercial and wild bird influenza viruses, a large sequence repository has been developed that will help prepare for future outbreaks, and hopefully allow us to predict which viruses are the most dangerous. Our work on using reverse genetics to make new vaccines provides great potential for vaccines use in the future and allows the more widespread use of DIVA (differentiate infected from vaccinated animals) vaccines. Several other vaccine technologies are being investigated to try and improve on the existing vaccines, either by making the vaccines more cost effective or by making them easier to administer. Several trade

embargoes from Mexico and Japan initiated research at SEPRL to look at the risk of virus spread in meat and to do pasteurization studies to demonstrate that current practices mitigated any risk of disease spread. These studies provided the scientific basis that was used to help lift these embargoes. Efforts to examine the pathogenesis of different avian influenza viruses have focused on the H5N1 viruses from Southeast Asia. These viruses are highly pathogenic for poultry, and some of them also pose a zoonotic threat. Numerous pathology studies have helped to identify host ranges and allow better risk assessment of these rapidly changing viruses.

TECHNOLOGY TRANSFER:
The real-time RT-PCR test for avian influenza has been transferred widely to both state and federal diagnostic laboratories. Additional test technology, including proficiency panels and neuraminidase N2 testing, have been transferred to APHIS, NVSL for their use.

PUBLICATIONS:

Cauthen, A.N., D.E. Swayne, S. Schultz-Cherry, M.L. Perdue, D.L. Suarez. Continued circulation in China of highly pathogenic avian influenza viruses encoding the hemagglutinin gene associated with the 1997 H5N1 outbreak in poultry and humans. 2000. Journal of Virology. 74: 6592-6599.

Dybing, J.K., S. Schultz-Cherry, D.E. Swayne, D.L. Suarez, M.L. Perdue. Distinct pathogenesis of Hong Kong-Origin H5N1 viruses in mice as compared to other highly pathogenic H5 avian influenza viruses. 2000. Journal of Virology 74:1443-50.

Perdue, M.L., and D.L. Suarez. Structural features of the avian influenza virus hemagglutinin that influence virulence. 2000. Veterinary Microbiology. 74:77-86.

Perdue, M. L., and B.Seal. "Impact of Avian Viruses". in *Viral Ecology*; 2000. C. Hurst, ed. pp 550-592. Academic Press.

Suarez, D.L. Evolution of avian influenza viruses. Veterinary Microbiology. 2000. 74:15-27.

Suarez, D.L., and S. Schultz-Cherry. Immunology of avian influenza: a review. 2000. Developmental and Comparative Immunology.24:269-283.

Suarez, D.L., and D.A. Senne. Sequence analysis of related low pathogenic and highly pathogenic H5N2 avian influenza isolates from United States live bird markets and poultry farms from 1983-1989. 2000. Avian Diseases. 44:356-364.

Swayne, D.E., and Suarez, D.L. Highly pathogenic avian influenza. 2000. Revue Scientifique et Technique 19: 463-482.

Swayne, D.E., M.L. Perdue, J.R. Beck, M. Garcia, D.L. Suarez. Vaccines protect chickens against highly pathogenic avian influenza in the face of genetic changes in field viruses over multiple years. 2000. Veterinary Microbiology. 74:165-172.

Swayne, D.E. Understanding the ecology and epidemiology of avian influenza viruses: implications for zoonotic potential. In: Emerging Infectious Diseases of Animals, eds C. Brown and C. Bolin. ASM Press, Washington D.C. Pp 101-130, 2000. (Book chapter)

Swayne, D.E., Beck, J.R., Kinney, N. Failure of a recombinant fowl poxvirus vaccine containing an avian influenza hemagglutinin gene to provide consistent protection against influenza in chickens pre-immunized with a fowl pox vaccine. 2000. Avian Diseases, 44:132-137.

Lee, C., Song,C., Lee,Y., Mo,I., Garcia,M., Suarez,D.L., Kim,S. Molecular and pathogenic characterization of Korean isolates of H9N2 avian influenza virus. 2000. Avian Diseases. 44.:527-535

Perdue, M.L., Suarez, D.L., Swayne, D.E. Avian influenza in the 90's. 2000. Poultry and Avian Biology Reviews. v.11. p.1-20.

Schultz-Cherry, S., Dybing, J.K., Davis, N.L., Williamson, C., Suarez, D.L., Johnston, R., Perdue, M.L. Influenza virus (A/HK/156/97) hemagglutinin expressed by an alphavirus replicon system protects chickens against lethal infection with Hong Kong-origin H5N1 viruses. 2000. Virology 278. p55-59.

Swayne, D.E., Beck, J.R., Zaki, S. Pathogenicity of West Nile virus for turkeys. Avian Diseases. 2000. 44: 932-937.

Wang X., Castro A.E., Castro M.D., Lu H., Weinstock D., Soyster N., Scheuchenzuber W., Perdue M. 2000. Production and evaluation criteria of specific monoclonal antibodies to the hemagglutinin of the H7N2 subtype of avian influenza virus. 2000. J Vet Diagn Invest 12:503-509.

Basler, C.F., Reid, A.H., Dybing, J.K., Janczewski, T.A., Fanning, T.G., Zheng, H., Salvatore, M., Perdue, M.L., Swayne, D.E., Garcia-Sastre, A., Palese, P., Taubenberger, J.K. Sequence of the 1918 Pandemic Influenza Virus Non-structural Gene (NS) Segment and Characterization of Recombinant Viruses Bearing the 1918 NS Genes. Proceedings of the National Academy of Science. 2001. 98:2746-2751.

Schultz-Cherry S., Dybdahl-Sissoko N., Neumann G., Kawaoka Y., Hinshaw V.S. Influenza virus NS1 protein induces apoptosis in cultured cells. 2001. J Virology. 75:7875-81

Perkins, L.E., Swayne, D.E. Clinicopathologic Aspects of Animal and Zoonotic Diseases of Bioterrorism: Avian Influenza. 2001. Clinics of North America. 21:549-590.

Perkins, L.E., Swayne, D.E. Pathobiology of A/Chicken/Hong Kong/220/97 (H5N1) Avian Influenza Virus in Seven Gallinaceous Species. 2001. Veterinary Pathology. 38:149-164.

Suarez D.L. Influenza of poultry and swine. Le Médecin Vétérinaire du Québec. 2000. v 30.p.187-192.

Suarez, D.L., Schultz-Cherry,S. The effect of eukaryotic expression vectors and adjuvants on DNA vaccines in chickens using an avian influenza model. Avian Diseases. 2000. v 44.p. 861-868.

Swayne, D.E., Beck, J.R., Perdue, M.L., Beard, C.W. Efficacy of Vaccines in Chickens Against Highly Pathogenic Hong Kong H5N1 Avian Influenza. Avian Diseases. 2001. v.45. p.355-365

Berinstein, A., B. Seal, D.L. Suarez. Heteroduplex mobility assay (HMA): its use for detection of new avian influenza virus (AIVs) variants. 2002. Avian Diseases. 46:393-400.

Perkins, L.E.L., Swayne D.E. Pathogenicity of Highly Pathogenic Avian Influenza Virus for Emus, Geese, Ducks and Pigeons. Avian Diseases. 2002. v. 46. p. 53-63.

Suarez, D.L., P.R. Woolcock, A.J. Bermudez, D.A. Senne. Isolation from turkey breeder hens of a reassortant H1N2 influenza virus with swine, human and avian lineage genes. 2002. Avian Diseases. 46:111-121.

Swayne, D.E., Suarez, D.L. Evolution and Pathobiology of Avian Influenza Virus Virulence in Domestic Birds. Dodet, B., Vicari, M., editors. John Libbey Eurotext, Paris. Emerging Diseases. Emergence and Control of Zoonotic Ortho- and Paramyxovirus Diseases. 2001. p. 35-42.

Swayne, D.E., Beck J.R., Smith C., Shieh W.J., Zaki S. West Nile Virus Causes Fatal Encephalitis and Myocarditis in Young Domestic Geese (*Anser anser domesticus*) Goose WNV. 2001. Emerging Infectious Diseases. v. 7. p. 751-753.

Tumpey, Terrence M., D.L. Suarez, L.E.L. Perkins, D.A. Senne, J.Lee , Y.. Lee , I. Mo , H. Sung , and D.E. Swayne. Characterization of a highly pathogenic H5N1 avian influenza A virus isolated from duck meat. 2002. J Virology. 76:6344-6355.

Perkins, L.E.L., Swayne, D.E. Susceptibility of laughing gulls (*Larus atricilla*) to H5N1 and H5N3 highly pathogenic avian influenza viruses. 2002. Avian Diseases. 46: 877-885.

Chen, H., Matsuoka, Y., Swayne, D., Chen, Q., Cox, N.J., Murphy, B.R., Subbarao, K. Generation and characterization of a cold-adapted influenza A H9N2 reassortant as a live virus vaccine candidate. 2003. Vaccine. 21: 1974-1979.

Perkins, L.E.L., Swayne, D.E., Varied pathogenicity of a Hong Kong-origin H5N1 avian influenza virus in four passerine species and budgerigars. 2003. Veterinary Pathology. 40: 14-24.

Subbarao, K., Chen, H., Swayne, D., Mingay, L., Fodor, E., Brownlee, G., Xu, X., Lu, X., Katz, J., Cox, N., Matsuoka, Y. Evaluation of a genetically modified reassortant H5N1 influenza A virus vaccine candidates generated by plasmid-based reverse genetics. 2003. Virology. 305: 192-200.

Swayne, D.E. and Halvorson, D.A. Influenza pp. 135-160. In Saif, Y.M., Barnes, H.J., Fadly, A., Glisson, J.R., McDougald, L.R. and Swayne, D.E. (eds.) Diseases of Poultry, 11th ed., Iowa State University Press, Ames, IA. 1231 pp. 2003. (Book Chapter)

Spackman, E. D. A. Senne, S. Davison and D. L. Suarez. Sequence Analysis of Recent H7 Avian Influenza Viruses Associated with Three Different Outbreaks in Commercial Poultry in the United States. 2003. Journal of Virology. 77:13399-13402.

Senne,D.A. D. L. Suarez, J. C. Pedersen and B. Panigrahy. Molecular and Biological Characteristics of H5 and H7 Avian Influenza Viruses Found in Live-bird Markets of Northeastern United States During 1994-2001. 2003. Avian Diseases. 47:898-904.

Bulaga, LL., L. Garber, D. Senne, T.J. Myers, R. Good, S. Wainwright, D.L. Suarez. Descriptive and Surveillance Studies of Suppliers to New York and New Jersey Retail Live Bird Markets. 2003. Avian Diseases. 47:1169-1176.

Bulaga, L.L., L. Garber, D.A. Senne, T.J. Myers, R. Good, S. Wainwright, S. Trock, D.L. Suarez. Epidemiologic and Surveillance Studies on Avian Influenza in Live-Bird Markets in New York and New Jersey, 2001. 2003. Avian Diseases. 47:996-1001.

Woolcock, P.R., D.L. Suarez, and D. Kuney. Low pathogenicity avian influenza virus (H6N2) in chickens in California, 2000-2001. 2003. Avian Diseases. 47:872-881.

Tumpey, T.M., D.L. Suarez, L.E.L. Perkins, D.A. Senne, J. Lee, Y. Lee, I. Mo, H. Sung, D.E. Swayne. Evaluation of highly pathogenic H5N1 avian influenza A virus isolated in duck meat. 2003. Avian Diseases. 47:951-955.

Swayne, D.E. D.L. Suarez, S. Schultz-Cherry, T.M. Tumpey, D.J. King, T. Nakaya, P. Palese, and A. Garcia-Sastre. Recombinat paramyxovirus type 1-Avian influenza-H7 virus as a vaccine for protection of chickens against influenza and Newcastle disease. 2003. Avian Diseases. 47:1047-1050.

Lee, C-W, D.A. Senne, and D.L. Suarez. Development of subtype-specific reference antisera by DNA vaccination of chickens. 2003. Avian Diseases. 47:1051-1056.

Yamnikova, S.S., A.S. Gambaryan, A.B. Tuzikov, N.V. Bovin, M.N. Matrosovich, I.T. Fedyakina, A.A. Grinev, V.M. Blinov, D.K. Lvov, D.L. Suarez, and D.E. Swayne. Differences between HA receptor-binding sites of avian influenza viruses isolated from “Laridae” and “Anatidae”. 2003. Avian Diseases. 47:1164-1168.

Suarez, D.L., Erica Spackman, and Dennis Senne. Update on Molecular Epidemiology of H1, H5 and H7 influenza infections in poultry in North America. 2003. Avian Diseases. 47:888-897.

Schultz-Cherry S, Koci M, Thompson E, Tumpey TM. Examining the cellular pathways involved in influenza virus induced apoptosis. 2003. Avian Dis. 47:968-71.

Hanson BA, Stallknecht DE, Swayne DE, Lewis LA, Senne DA. Avian influenza viruses in Minnesota ducks during 1998-2000. 2003. Avian Dis. 47:867-71.

Perkins L.E., Swayne D.E. Comparative susceptibility of selected avian and mammalian species to a Hong Kong-origin H5N1 high-pathogenicity avian influenza virus. 2003. Avian Diseases 47:956-967.

Beck J.R., Swayne D.E., Davison S., Casavant S., Gutierrez C. Validation of egg yolk antibody testing as a method to determine influenza status in white leghorn hens. 2003. Avian Diseases. 47:1196-1199.

Suarez,D.L., D.A. Senne, J. Banks , I.H. Brown, S.C. Essen, C.W. Lee, R.J. Manvell, , C. Mathieu-Benson V. Moreno, J. Pedersen, B. Panigrahy, H. Rojas, E. Spackman, and D.J. Alexander. Recombination Resulting in Virulence Shift on Avian Influenza Outbreak, Chile. 2004. Emerging and Infectious Diseases. 10:693-699.

Jones Y.L., Swayne D.E. Comparative pathobiology of low and high pathogenicity H7N3 Chilean avian influenza viruses in chickens. 2004. Avian Diseases. 48:119-128.

Lee, C.W. and D.L. Suarez. Application of real-time RT-PCR for the quantification and competitive replication study of H5 and H7 subtype avian influenza virus. 2004. Journal of Virological Investigation. 119:151-158.

Swayne, D.E., D.L. Suarez, E. Spackman, T.M. Tumpey, J.R. Beck, D. Erdman, P. E. Rollin and T. G. Ksiazek. SARS-Coronavirus Does Not Cause Disease or Infection in Experimentally Inoculated Domestic Poultry. 2004. Emerging and Infectious Diseases. 10:914-916.

Tumpey T.M., Garcia-Sastre A., Taubenberger J.K., Palese P., Swayne D.E., Basler C.F. Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus. 2004. PNAS. 101:3166-3171.

Lee, C.-W., D. Senne, J. A. Linares, P. Woolcock, D. Stallknecht, E. Spackman, D.E. Swayne, and D.L**.** Suarez. Characterization of Recent H5 Subtype Avian Influenza Viruses from U.S. Poultry. Avian Pathology. (In press)

Lee, C.W., D.A. Senne, and D.L. Suarez. Generation of reassortant influenza vaccines by reverse genetics that allows utilization of a DIVA (Differentiating Infected from Vaccinated Animals) strategy for the control of avian influenza. Vaccine. (In press)

Richt, J, K.M. Lager, D.F. Clouser, E. Spackman, D.L. Suarez, K. Yoon. Real-time RT-PCR assays for the detection and differentiation of North American swine influenza viruses. Journal of Veterinary Diagnostic Investigation. (In press)

Lee, C.W., D.A. Senne, and D.L. Suarez. Effect of Vaccine Use in the Evolution of Mexican-lineage H5N2 Avian Influenza Virus. Journal of Virology (In press)

Southeast Poultry Research Laboratory

CRIS Title: Mucosal immunization to protect poultry against avian influenza.
CRIS Number: 6612-32000-040-00D
Scientists: Vacant, Swayne DE, Suarez DL
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PROJECT OBJECTIVES:

Avian influenza (AI) viruses have been circulating periodically in domestic poultry over the past 100 years. Wild waterfowl and shorebirds are the reservoir for all identified AI virus subtypes and although chickens and turkeys are not natural host species, these viruses can routinely crossover from the wild-bird reservoir to poultry. Although the incidence of highly pathogenic AI viruses are relatively rare, less pathogenic strains of these viruses more often circulate through poultry flocks and are responsible for significant morbidity, mortality and production losses. The devastation of low pathogenic (LP) H7N2 avian influenza (AI) in chickens and turkeys has been apparent in the eastern U.S. in 2001 and 2002 resulting in depopulation of 4.7 million commercial turkeys and chickens and is estimated to have cost the poultry industry approximately $130 million. In addition, outbreaks have occurred in live poultry markets of New England. In 2004, a limited outbreak of highly pathogenic (HP) H5N2 AI occurred in Texas.

Traditional parenteral vaccination has provided some success at providing protection against disease but is less effective in protecting against infection. This is thought to be due in part to the observation that systemic vaccination is a poor inducer of mucosal immunity and therefore organisms can invade the host before the systemic immunity can impede the infection. Because AI viruses invade mucosal surfaces, emphasis should be placed on vaccines that induce strong mucosal immunity. Thus, the main objective of this CRIS will be to develop mucosal vaccines in an attempt to induce greater immune protection. The development of new vaccine approaches for controlling such emerging/reemerging pathogens can be of great importance to the profitability of the poultry industry.

OVERALL PROJECT ACCOMPLISHMENTS:

Accomplishments over the life of the project included publication of techniques for the identification of avian immunoglobulins. These techniques have included the development of a monoclonal antibody (mAb) to IgA and has improved the sensitivity of measuring IgA antibody titers in immune samples. The mAb has also reduced the background levels observed with commercially available reagents. Other accomplishments for this CRIS included: (1) improved biological reagents needed to measure IgG antibody responses in chickens and turkeys, (2) development of an ELISPOT assay to measure antibody secreting cells in various tissues, (3)

identification of mucosal adjuvants for use in inactivated poultry vaccines, and (4) develop and evaluation of inactivated AI and a recombinant Newcastle disease virus vaccine with AI hemagglutinin gene insert. Research at Southeast Poultry Research Laboratory has also demonstrated that the route of vaccine influenced the optimal mucosal response in white leghorn chickens. The identification of optimal mucosal vaccine routes(s) is crucial for improving mucosal immune response(s) to avian influenza viruses that infect poultry. Protocols were established for vaccines and vaccination to protect turkeys from LPAI viruses.

IMPACT:
The new assays provide a mechanism to assess the effectiveness of vaccines to protect poultry against AI and other respiratory and intestinal pathogens at the site of pathogen growth and injury. The vaccine technology improvements have produced vaccines for salmonella and AI that gives improved protection against their respective diseases than produced by existing commercial products. One of these will decrease the cost of vaccination for AI because it can be given by spray and not injection. This information will be used to develop efficacious vaccines for stimulating protective immunity against AI and possibly other respiratory pathogens that affect poultry. Furthermore, the identification of immune effectors mediating protection indicates ways to maximize protective immune responses in poultry.

TECHNOLOGY TRANSFER:
No technology transfers.

PUBLICATIONS:

Chaubal, L. H., Holt, P. S. Characterization of motility and identification of flagella proteins in the avian pathogen Salmonella pullorum. American Journal of Veterinary Research. 2000. v. 60. p. 1322-1327.

Katz, J.M., Lu, X., Frace, M. Morken, T., Zaki, S. R., Tumpey, T.M. Pathogenesis of and Immunity to Avian Influenza A H5 viruses. Biomedicine Pharmacotherapy. 2000. v.54. P.178-187.

Tumpey, T.M., Lu, X., Morken, T., Zaki, S. R., Katz, J.M. Depletion of lymphocytes and diminished cytokine production in mice infected with a highly virulent influenza A virus isolated from humans. Journal of Virology. 2000. v.73. P.6105-6116.

Tumpey, T.M., Renshaw, M., Clements, J., Katz, J.M. Mucosal delivery of inactivated influenza vaccine induces B cell dependent heterosubtypic protection against a lethal influenza A H5N1 virus. Journal of Virology. 2001. v.75. p. 5141-5150.

Lu, X., Tumpey, T. M., Renshaw, M., Hu-Primmer, J., Katz, J. Immunity to influenza A H9N2 viruses induced by infection and vaccination. Journal of Virology 2001. v.75. P.4896-4901.

Tumpey, T. M., Renshaw, M., Clements, J., Katz, J. Mucosal delivery of inactivated influenza A H3N2 vaccine induces B cell-dependent heterosubtypic protection against a lethal influenza A H5N1 virus. Journal of Virology. 2001. v.75. p.5141-5140.

Sambhara, S., Kurichh, A., Miranda, R., Tumpey, T. M., Renshaw, M., Arpino, R., Kandil, A., James, O., Underdown, B., Katz, J., Burt. D. Heterosubtypic immunity against human influenza A viruses including recently emerged avian H5 and H9 viruses induced by Flu-ISCOM vaccine in mice requires both CTL and macrophage function. Cellular Immunology. 2001. v.211. p.143-153.

Tumpey, T. M., D. L. Suarez, L. E. L. Perkins, D. A. Senne, J. Lee, Y. Lee, I.-P. Mo, H-W Sung., Swayne, D.E. Characterization of a highly pathogenic H5N1 avian influenza A virus isolated from duck meat. Journal of Virology. 2001. v.76. p.6344-6355.

Epstein, S.L., Tumpey, T. M., Misplon, J.A., Lo, C-Y, Cooper, L.A., Subbarao, K., Renshaw, M., Sambhara, S., Katz, J.M. DNA vaccine expressing conserved influenza virus proteins confers partial protection against H5N1 challenge in mice. Emerging Infectious Diseases. 2002. V.8. p.796-801.

Geiss, G.K., M. Salvatore, Tumpey, T. M., V. S. Carter, W. Xiuyan, C. F. Basler, A. Mikulasova, J. K. Taubenburger, R. E. Bumgarner, P. Palese M.G. Katze, García-Sastre, A. Cellular transcriptional profiling in influenza A virus-infected lung epithelial cells: The role of the nonstructural NS1 protein in the evasion of the host innate defense and its potential contribution to pandemic influenza. Proceedings of the National Academy of Sciences USA. 2002. v.99. p.10736-10741.

Tumpey, T.M., García-Sastre, A., Mikulasova , A., Taubenberger, J.K., Swayne, D.E., Palese, P., Basler, C.F. Existing antivirals are effective against influenza viruses with genes from the 1918 pandemic virus. 2002. Proceedings of the National Academy of Science. v.99. p.13849-13854.

Tumpey, T. M., Fenton, R., Molesworth-Kenyon, S, Oakes, J.E., Lausch, R.N. Role for MIP-2, MIP-1 and IL-1 in delayed type hypersensitivity response to viral antigen. Journal of Virology. 2002. v.76. p.8050-8057.

Tumpey, T. M., García-Sastre, A., Mikulasova, A., Taubenburger, J., Swayne, D.E., Palese, P., Basler, C.F. Cellular transcriptional profiling in influenza A virus-infected lung epithelial cells: The role of the nonstructural NS1 protein in the evasion of the host innate defense and its potential contribution to pandemic influenza. Proceedings of the National Academy of Sciences USA. 2002. v.99. p.13849-13854.

Tumpey, T.M., Suarez, D.L., Perkins L.E.L., Senne, D.A., Lee Y. Lee, I-P. Mo, Swayne, D.E. Characterization of a highly pathogenic H5N1 avian influenza A virus isolated from duck meat. Journal of Virology. 2002. v.76. p.6344-6355.

Chen, H., Matsuoka, Y., Swayne, D., Chen, Q., Cox, N.J., Murphy, B.R., Subbarao, K. Generation and characterization of a cold-adapted influenza A H9N2 reassortant as a live virus vaccine candidate. Vaccine. 2003. v.21. p.1974-1979.

Swayne, D.E., Suarez, D.L., Schultz-Cherry, S., Tumpey, T., King, J.D., Nakaya, T., Palese, P., Garcia-Sastre, A. Recombinant paramyxovirus type 1-avian influenza-H7 virus as a vaccine for protection of chickens against influenza and Newcastle disease. Avian Diseases. 2003. v. 47. p.1047-1050.

Kapczynski, D.R., Tumpey, T. M. Development of a virosome vaccine for Newcastle disease virus. Avian Disease. 2003. v.47. p.578-87.

Matsuoka, Y., Chen, H., Cox, N., Subbarao, K., Beck, J.R., Swayne, D.E. Safety evaluation in chickens of candidate human vaccines against potential pandemic strains of influenza. Avian Diseases. 2003. v.47. p.926-930.

Subbarao, K., Chen, H., Swayne, D., Mingay, L., Fodor, E., Brownlee, G., Xu, X., Lu, X., Katz, J., Cox, N., Matsuoka, Y. Evaluation of a genetically modified reassortant H5N1 influenza A virus vaccine candidates generated by plasmid-based reverse genetics. Virology. 2003. v.305. p.192-200.

Swayne, D.E. Vaccines for list A poultry diseases: emphasis on avian influenza, pp. 201-202. In Brown, F. and Roth, J. (eds.). Vaccines for OIE list A and Emerging Animal Diseases. Developments in Biologicals. 2003. v. 114. p.201-212.

Tumpey, T.M., Kapczynski, D.R., Horton, R, Swayne, D.E. Evaluation of a commercial avian influenza (H7N2) vaccine for protection in turkeys against an avian influenza virus (H7N2) isolated from turkeys in Virginia during 2002. Avian Diseases. 2004. v. 48. p.167-176.

Southeast Poultry Research Laboratory

Project Title: Development and Validation of Rapid Diagnostic Tests for Avian Influenza and Newcastle Disease
CRIS Number: 6612-32000-041-00X
Scientists: Swayne D., Suarez DL, King DJ, Spackman E, Kapczynski, D
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PROJECT OBJECTIVES:

Highly pathogenic avian influenza (AI) and Newcastle disease (ND) are severe systemic, highly transmissible diseases of poultry that cause high mortality rates and severe economic losses. Traditionally, diagnosis has been slow, requiring virus isolation and identification. This delayed diagnosis has hampered efforts for early detection and elimination, resulting in devastating natural outbreaks. Complicating diagnosis is the existence of low virulent forms of AI and avian paramyxovirus type 1 (synonymous with Newcastle disease virus) viruses that can mutate to become highly pathogenic avian influenza and Newcastle disease viruses, respectively. These low virulent forms must be differentiated from common respiratory diseases. The sources of AI and ND viruses include domestic poultry and wild birds, but genetic analysis of diverse virus isolates across time, geographic location and bird species have not been accomplished. Genetic analysis of AI and ND viruses has been non-systematic, leaving critical gaps on genetics in our knowledge base.

To develop a rapid diagnostic test for these two diseases, real time reverse transcriptase polymerase chain reaction (RRT-PCR - a biotechnology test to detect genes), technology will be developed to detect the viruses in birds and differentiate these viral infections from "look-a-like" respiratory diseases. Initially, probes will be designed based on the current AI and ND virus sequences, and tested in an RRT-PCR test. Second, surveillance in wild and domestic birds will yield new genetically diverse AI and ND virus isolates to test the sensitivity and specificity of the RRT-PCR probes. Various genes from genetically diverse isolates will be sequenced and analyzed to improve probe design and the specificity and sensitivity of RRT-PCR test. Furthermore, the sequences of diverse viral isolates will help build molecular epidemiological maps for future determination of the source of outbreak viruses. Third, RRT-PCR tests will be validated in experimental studies. Fourth, the RRT-PCR tests will be validated in the field in the U.S. and foreign nations.

OVERALL PROJECT ACCOMPLISHMENTS:

Rapid RT-PCR (RRT-PCR) test were developed and validated in laboratory and the field to diagnosis type A influenza virus (avian influenza virus) and differentiate H5 and H7

hemagglutinin subtypes in diagnostic samples. RRT-PCR tests were developed and validated in the laboratory and field to diagnose Newcastle disease.

IMPACT:
The AI RRT-PCR tests were used in the 2002 Virginia H7N2 AI outbreak and 2002 Live Poultry Markets to make AI diagnoses in less than 24 hrs. The NDV RRT-PCR test was used in California during 2003. These tests have become the National Standard tests for diagnosis of AI and ND viruses at the National Veterinary Services Laboratories and have been distributed to National Animal Health Laboratory System for use at the State Veterinary Diagnostic Levels to get rapid diagnosis of AI and ND.

TECHNOLOGY TRANSFER:
The Southeast Poultry Research Laboratory (SEPRL) has been on the leading edge of use of real time PCR testing as a diagnostic test for viral pathogens of poultry with its work on avian influenza virus and Newcastle disease virus. Through collaborations with APHIS, the rapid diagnostic test for avian influenza and Newcastle Disease was validated and adopted by APHIS as a principle test for the detection of these agents from field samples. These tests has already been adopted by many state veterinary diagnostic laboratories with interest from others, and they are official tests for the National Animal Health Laboratory Network. SEPRL has been instrumental in the technology transfer to these laboratories by providing reference reagents, training, coded test panels and other resources materials for the implementation of the technology in each laboratory.

PUBLICATIONS:

Spackman, E., Senne, D.A., Myers, T.J., Bulaga, L.L., Garber, L.P., Perdue, L.P., Lohman, K., Daum, L.T., Suarez, D.L. Development of a real-time RT-PCR assay for type A influenza and the avian H5 and H7 hemagglutinin subtypes. 2002. Journal of Clinical Microbiology. 40:3256-3260.

Spackman,E., D.A. Senne, L.L. Bulaga, T.J. Myers, M.L. Perdue, L.P. Garber, K. Lohman, L.T. Daum, and D.L. Suarez. Development of real-time RT-PCR for the detection of avian influenza virus. 2003. Avian Diseases. 47: 1079-1082.

Suarez, D.L., E. Spackman, D. Senne, L. Bulaga, A. C. Welsch, K. Froberg. The Effect of Various Disinfectants on Detection of Avian Influenza Virus by Real Time RT-PCR. 2003. Avian Diseases. 47:1091-1095.

Spackman, E., D.A. Denne, L.L. Bulaga, S. Trock, and D.L. Suarez. Development of a multiplex real-time RT-PCR as a diagnostic tool for avian influenza. 2003. Avian Diseases. 47: 1087-1090.

Wise MG, Suarez DL, Seal BS, Pedersen JC, Senne DA, King DJ, Kapczynski DR, Spackman E. Development of a real-time reverse-transcription PCR for detection of Newcastle disease virus RNA in clinical samples. 2004. Journal of Clinical Microbiology. 42:329-38.

Spackman, E., and D.L. Suarez. Use of a novel virus inactivation method for a multi-center avian influenza real-time RT-PCR proficiency study. Journal of Veterinary Diagnostic Investigation. (In press)

Southeast Poultry Research Laboratory

Project Title:	Development of environmental and genomic strategies to control Marek's disease
CRIS Number:	6612-32000-043-00D
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PROJECT OBJECTIVES:
Marek's disease is a cancer of the lymphocytes in chickens caused by a herpesvirus. Vaccines have been used since the 1960's to prevent and/or control development of the cancer. Such vaccines have been successful but new strains of the virus have arisen through virulence shifts that have overcome vaccine protection. The objectives of this project are: 1) development of environmental control systems that will reduce field exposure of chickens to the Marek's disease virus and result in reduced incidence of Marek's disease, and 2) development of a comparative genomics program that will lead to the identification of predictors of virulence shifts. Initially, objective one will be approached by developing and testing high voltage electrostatic space charge systems (ESCS) for use in chick rearing areas and poultry houses to reduce feather dander containing the Mareks' disease virus. ESCS systems designed at SEPRL has been shown to reduce dust in hatching cabinets and animal rooms. Feather dander is a major source of dust particles. Marek's disease is transmitted because the virus is shed in high quantities of feather dander. Impact of ESCS on reducing environmental load of Marek's disease virus and transmission of Marek's disease virus will be determined. A genomics approach will be used to identify viral genes responsible for shifts in virulence of field MDV which will assist in vaccine development.

OVERALL PROJECT ACCOMPLISHMENTS:
None. New project initiated in late 2003.

IMPACT:
None. New project

TECHNOLOGY TRANSFER:
None.

PUBLICATIONS:
None.

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PROJECTS

Animal Parasitic Diseases Laboratory (APDL)

- Genomic, Proteomic and Immunological Approaches to Control Avian Coccidian Parasites
- Strategies to Control Swine Parasites Affecting Food Safety
- Biosystematics and Biodiversity of Pathogens and Parasites
- Biology and Epidemiology of Neospora Caninum and Related Protozoa

Bovine Functional Genomics Laboratory (BFGL)

- Improving Milk Quality by Reducing Mastitis in Dairy Cattle
- Immunological and Genetic Basis of Resistance to Parasites of Cattle

Animal Parasitic Diseases Laboratory (APDL)

Project Title: Genomic, Proteomic and Immunological Approaches to Control Avian Coccidian Parasites
CRIS Number: 1265-31320-070-00D
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PROJECT OBJECTIVES:

Coccidiosis is a ubiquitous intestinal protozoan infection of poultry which seriously impairs the growth and feed utilization of infected animals. In poultry, coccidiosis has been particularly problematic where annual losses of over $600 million per year have been sustained by the U.S. poultry industry alone. Each of the more than 30 billion chickens raised annually worldwide is at risk for coccidiosis. Coccidiosis in chickens is caused by at least seven distinct species of obligate intracellular parasites of the genus Eimeria. The pathology of coccidiosis is caused by the rapid replication of the parasite during asexual reproduction in the cells of the gut, resulting in diarrhea, weight loss, and anorexia. Several notable problems currently exist for effective control of coccidiosis: 1) Disease resistant strains have been emerging very rapidly worldwide. Therefore the effectiveness of existing pharmaceutical therapeutics have been decreasing steadily over the last decade. This problem is compounded by our lack of understanding of the mechanism(s) that confer(s) drug resistance and how the drug resistant strains are propagated. 2) Knowledge of the nature of protective immunity elicited by natural infection is limited. Vaccination against Eimeria induces species-specific immunity, and is the basis for increased utilization of live oocyst vaccines in broiler production. However, strains of some species (a particular problem with E. maxima) are poorly cross-protective, reducing the effectiveness of live vaccines. 3) The limited number of existing recombinant vaccines do not offer cross-protection against all seven species of Eimeria that infect chickens. 4) Additionally, there is worldwide consumer as well as governmental effort to reduce the use of feed additive pharmaceuticals for animals destined for human consumption, further driving the need for more efficacious vaccines. Our long-term goal is to develop new cost-effective strategies that will control coccidiosis for the poultry producer. To accomplish this, we are conducting research through a multidisciplinary approach using immunology, biochemistry, cellular and molecular biology and functional genomics and proteomics to 1) identify host genes and traits which confer protective immunity against all Eimeria species and use this information to develop marker-assisted selection strategy for commercial broiler industry, 2) determine the immunological pathways that provide innate and acquired host resistance, and understand the early physiological host responses associated with the immune response and pathology, 3) develop new vaccine strategies for induction of protective mucosal immunity, 4) identify parasite genes and gene products that are related to growth and development and that may play roles in pathology and drug resistance. These genes may prove to be targets for new vaccines and chemotherapy. 5) understand the physiology of parasite development through studies of *Eimeria* parasites in cell culture, 6) modulate infections through use of non-pharmaceutical feed additives, and 7)

understand the genetic mechanisms which may drive antigenic diversity in the parasites. Successful completion of these studies will enhance our understanding of *Eimeria* biology and genetics, the development of host immunity to parasite invasion, innate disease resistance to coccidiosis, and will assist in the development of practical, multifaceted, effective control strategies against *Eimeria* sp.

OVERALL PROJECT ACCOMPLISHMENTS:

1. *Identification of Eimeria genes and gene product which are involved in immunity and parasite development.* We have targeted new structural, stress-related, and developmentally regulated parasite proteins associated with several Eimeria species as potential protective antigens, produced recombinant parasite proteins that have been tested using new methods of vaccine delivery including various viral vector systems as well as DNA vaccines. Subtracted cDNA libraries from E. tenella oocysts were produced and over 500 ESTs representing transcripts whose expression is differentially regulated between sporulated and unsporulated oocysts were identified. Analysis of expression of several of these transcripts via real-time PCR methods confirmed that these transcripts are differentially expressed. The full-length sequences of some candidates are being obtained, and will be tested as potential targets for vaccine development. In addition, analysis of ESTs from E. acervulina sporozoites and merozoites has resulted in identification of four full-length candidate transcripts that will also be tested in vaccine development. These candidates include a serine protease inhibitor, macrophage inhibitory factor, a member of a family of surface proteins, and peroxiredoxin. All of these molecules may be essential to parasite function, and may serve as good vaccine targets. Recent success in characterizing genes that are important during sporulation enhanced our knowledge of parasite development and will facilitate the development of new therapeutics that would inhibit the formation of the infectious oocyst therefore preventing infection of the host. A major ooccyst protein was identified and characterized during sporulation of E. tenella. This protein can serve as an important marker of development in Eimeria species. Further studies to examine the changes in other oocyst proteins that occurs during sporulation using a combination of mass finger-printing techniques and n-terminal sequencing to identify proteins characterized on 2D gels and the use of real-time PCR and micro-array system to examine the quantitative changes in proteins during maturation of the oocysts will complement our ongoing efforts to develop coccidia vaccine. Investigation of enzyme changes during sporulation of E. tenella will also lead to the identification of key enzymes associated with sporulation and sporozoite invasion of host cells and determine if sporozoite proteins are synthesized de novo or are incorporated from unsporulated oocysts. In efforts to find Eimerian metabolic systems that would be suitable targets for control programs, we identified one enzyme of the biotin-containing acetyl coA carboxylase complex (ACC1). This complex encompasses the initial steps in synthesis of fatty acids, requisite components of cell membranes, and has been found to be susceptible to inhibition by acetoxyphenoxypropionate ("fop") type herbicides in both *Plasmodium sp* and *Toxoplasma sp.* These herbicides inhibit growth of these parasites in vitro. ACC1 activity is present in apicoplast organelles in these two parasites. However, Eimeria growth and reproduction was not inhibited by two "fop" herbicides when fed to coccidia-infected chickens. We have found that the presence of

apicoplast organelles in Eimeria is questionable, and that although a genetic sequence similar to the acetyl coA carboxylase of Toxolasma is present in the E. tenella genetic data base (Sanger, UK), there does not seem to be a leader sequence that would target it to an apicoplast. The complex may be in the cytoplasm, or confined to Golgi vesicles. The presence of the ACC1 complex transcripts is being confirmed by sequencing of RT-PCR products amplified from cDNA of E. tenella sporulated oocysts, and probes are being developed to facilitate its localization histologically. These findings reveal an exciting, fundamental metabolic difference between *Eimeria sp* and other apicomplexa that may be exploitable for new control strategies. Proteins in developmental stages of Eimeria species have been characterized. An important antigen of unknown function previously reported in E. acervulina was identified to act as an actin binding protein, profilin. This was the first report of profilin from apicomplexa. The structure of this protein was deduced from the E. tenella genomic data base. The protein is conserved in all species of Eimeria studied and is unique to the Apicomplexans. Profilin transcripts and the subsequent protein are expressed at all stages tested

2. *Provided new information on coccidia proteins, recombinant vectors, and mucosal delivery methods that will facilitate the development of novel vaccine strategies for avian coccidiosis for the poultry industry*. Vaccine trials using Eimeria immunogenic recombinant vaccines have clearly demonstrated that subunit, recombinant, and DNA vaccines can elicit protective immunity. Identification of new structural and metabolic Eimeria proteins, other stress-related and developmentally regulated Eimeria proteins and development of various novel vaccination strategies using attenuated, recombinant viral vectors, recombinant antibody or various delivery methods such as gene gun, in ovo injector have demonstrated the feasibility of eliciting protective intestinal immune responses. A systematic proteomic approach was implemented to determine the protein compliment of developmental stages of Eimeria and determine the interaction of these proteins with the host immune system. Pure, live oocysts strains have been characterized for drug sensitivity and cross protection potential. Immuno-stimulatory complexes (ISCOMS) have been investiaged as antigen delivery systems in vaccination protocols for avian coccidiosis. Saponins from plants native to Kazakstan were evaluated for low toxicity and ability to form ISCOMs. The best saponins were used to prepare ISCOMs containing crude coccidia antigens. Immunization with these ISCOMS significantly stimulated cellular and humoral immunity in mice and provided significant protection to re-infection in immunized chicken. Recombinant Eimeria antigens are being expressed and will be incorporated into ISCOMS for further vaccination trials. To address problems in cross protection studies, or in trials comparing efficacies of various anticoccidial treatments, we have developed a protective index that takes into consideration both gross aspects (weight gain and lesion score) and metabolic indicators (plasma carotenoids and $NO_2^-+NO_3^-$) of pathology during coccidia infections. Recent cross protection studies with several E. maxima strains indicate that parasite fecundity is a major factor in eliciting protective immunity. Thus, genes that regulate the reproductive capacity of Eimeria sp. will be important targets for control of avian coccidia infections. Further studies on the role of chicken cytokines as adjuvant in DNA vaccine will enhance our understanding of how the chicken cytokine modulate local immunity to parasites. The

results of our studies clearly document the role of various immunomodulators such as chicken cytokines, saponins as adjuvants that should provide additional impetus for further investigation of their biological activities at the molecular and immunological levels. These results provide important background information from which novel vaccine technology can be developed for coccidiosis control.

3. *Developed a large scale screening system for testing drug and vaccine sensitivities of field isolates of Eimeria and host immune responses to vaccination.* A new method to conduct a large scale evaluation of current anti-coccidial vaccines and drugs, and to assess the protective host immune response to vaccination has been developed and is being evaluated as way to create efficient management of avian coccidiosis. As evidence of the importance of this work, several CRADAs have been initiated with poultry and pharmaceutical companies to evaluate drug sensitivity of field strains of avian coccidia. Furthermore, the lack of appropriate immunological reagents to study the poultry immune responses is a major limiting factor for the progress of poultry disease research. Industry support was obtained to test field strains of Eimeria for sensitivity to common anti-coccidial drugs or live oocysts vaccines from US poultry producers. To continue the investigation on the role of cytokine as genetic adjuvant, a National Research Initiative grant was awarded to characterize and clone major chicken lymphokines (IFN-γ, IL-15, IL-2, IL-16 and IL-17) that are associated with protective host immune responses to coccidiosis to develop enzyme-linked immunosorbent assays (ELISA) to evaluate the host immune response to vaccination on a large scale.

4. *Identified DNA and physiological markers that are associated with pathology and immune protection in commercial meat-type chickens.* . Genetic mapping using commercial chicken strains has found quality trait loci (QTL) markers on chicken chromosome 1 associated for resistance to coccidia, weight gain, and oocyst production. The locus identified as "LEI0101" decreased parasite production 3000 times in broiler chicken families. This was the first description of an association of chicken genetics with coccidiosis resistance and this finding will be used by the poultry industry for marker assisted selection (MAS) of breeding stocks. This research provides the groundwork to develop novel strategies of improving the genetic capacity of chickens for enhanced resistance to coccidiosis and was the basis for a new NRI Grant to develop a practical marker-assisted selection strategy for avian coccidiosis in commercial broilers. New knowledge concerning the genetics and physiology controlling the interaction of the host and parasite are required to develop markers that can be used to optimize new treatment strategies for avian coccidiosis. Hormonal changes associated with appetite suppression and reduced feed intake have been identified, linking for the first time early host pathology to neuroendocrine changes. Additionally 3-methylhistidine has been found to be a metabolic marker associated with muscle protein breakdown and with reduced feed intake. infections. Availability Genetic mapping (definition of DNA markers) for resistance to coccidiosis was initiated through the Award of a Fund for Rural America (FRA) and extended through a National Research Initiative grant which has also allowed the construction of the cDNA library. The first normalized chicken intestinal cDNA library containing >38,000 gene sequences has been developed. Availability of chicken

intestinal genome database will facilitate the identification of the nature of host genes controlling nutrition, immunity, physiology, growth and disease resistance which are important in the studies on the mechanisms of growth and development. Development of functional genomics technology led to the development of a Trust agreement entitled "Application of Functional Genomics to Develop Novel Control Strategies Against Poultry Diseases". Chicken intestinal cDNA microarray will lead to identification of host genes which control innate and acquired immune responses to coccidiosis and facilitate the development of novel immunological control strategies for coccidiosis. Cooperative work with Growth Biology Cooperative work with Growth Biology Lab showed a high coincidence of acute reduced feed intake and acute increase in the appetite suppressing hormone ghrelin at 4 days post infection with the duodenal parasite, E. acervulina. These changes were also coincidental with significant reduction in plasma levels of leptin, IGF-1 and T3, and a significant increase in pancreatic glucagon. These hormonal changes were followed by significant weight gain reduction. Weight gain and feed conversion are the most economically important production parameters to poultry producers. These studies are the first to show a significant impact of coccidia infections of the chick small intestine on the host neuroendocrine system leading to multiple hormonal changes, and to reveal the direct relationship they likely have with feed intake weight gain reduction and muscle protein accretion. Because these hormonal effects occur before gross pathology is evident, a shift in research emphasis to early host-parasite interactions may uncover the primary biochemical and immunological host responses. This, in turn, may aid in defining the important genes associated with resistance and susceptibility to coccidiosis. We have found that this weight gain decrease is associated with muscle protein break-down as assessed by increased levels of plasma 3-methylhistidine. The ratio of reduced/oxidized glutathione decreases significantly in blood, intestinal mucosa and liver at 6 days post infection during primary infections with both *E. acervulina* and *E. tenella*. Reduction in the red/ox ratio is an indicator of oxidative stress, and is reported to play an important signaling role in the development of cellular immunity.

5. *Developed molecular diagnostic method to identify Eimeria field strains and investigate underlying molecular mechanisms of antigenic diversity of field Eimeria species.* In order to implement precision agriculture techniques in determining species of coccidia in the field, polymerase chain reaction (PCR)-based techniques have been developed and used to detect the seven recognized species of Eimeria. This method proved to be useful for the industry as well as customers since it provides a fast and accurate detection of coccidian parasites. This technique conserves time and well as resources since determination of species composition by microscopic examination takes no less than 3 weeks. Microscopic examination is also fraught with error given the similar morphology of these species. The PCR method is based on specific amplification of single Eimeria species. The test, which can be completed in a single day, utilizes 7 different pairs of oligonucleotide primers that are directed to species-specific regions of ribosomal DNA. The validity of this technique was confirmed by the testing of several litter samples from nearby farms. Additionally, elucidation of underlying molecular mechanisms responsible for generation of genetic diversity of Eimeria species is important and aid in development of recombinant vaccines. Among the 3 major species of poultry coccidia, E. tenella, E.

acervulina, and E. maxima, E. maxima is believed to be more antigenically diverse than the other two species, and immunization of chickens with one strain of E. maxima does not always protect against infections with other strains of the same species. This is important for producers of live Eimeria oocyst vaccines which face the prospect that one strain of E. maxima may not be sufficient for protecting chickens against coccidiosis. We have observed that while the genetic diversity of ribosomal DNA in E. tenella and E. acervulina is fairly small, E. maxima displays significantly greater amounts of sequence diversity. The data collected so far suggests that the genetic organization of ribosomal DNA in E. maxima may be substantially different than in the other two species. It is likely that in the genome of E. maxima multiple ribosomal DNA loci exist on different chromosomes, while in E. tenella and E. acervulina the more conventional organization of the ribosomal DNAs is seen, with all ribosomal DNA being contained in a single closely linked locus. This data provides the first genetic evidence that E. maxima may be fundamentally different from other Eimeria species so far studied, and may lead to the understanding of the mechanisms behind the antigenic diversity present in this species.

6. *Immunoenhancement of intestinal innate immunity using dietary supplementation, probiotics, CpG oligodinucleotides, cytokines and recombinant antibodies.* Due to increasing concerns over the use of drugs in poultry production, alternative methods need to be developed. Our studies have demonstrated that cytokines, probiotics, CpG oligodinucleotides (ODN), vitamins and recombinant antibodies can enhance intestinal immunity against coccidiosis. For example, IFN-γ is an important cytokine that activates macrophages and is produced by activated lymphocytes in the intestine following coccidiosis. Role of IFN-γ production during coccidiosis has been well studied using a quantitative RT-PCR and recently using gene expression profiling. In E. tenella-infected chickens, IFN-γ transcripts were detected in the spleens, cecal tonsils, and IEL following the primary and the secondary infections with *E. tenella*. Multiple intramuscular injections of chIFN-γ on one day prior to, and two and four days after infection with *E. acervulina*, conferred significant protection as measured by body weight loss and oocyst shedding in white leghorn chickens. The treatment of chicken cells with the recombinant chIFN-γ inhibited the intracellular development of E. tenella without affecting the sporozoite invasion of host cells. Another cytokine, interleukin-2 (IL-2) plays an important role in the function of the immune system and is a potent growth factor for a variety of cell types including T-cell differentiation, B-cell development and NK cell activation. Co-injection of the IL-2 gene with the 3-1E coccidia gene enhanced the host response to the recombinant vaccination. Recently we have assessed the effects of directly injecting genes encoding the chicken cytokines IL-1β, IL-2, IL-8, IL-15, IFN-α, IFN-γ, TGF-β4, or lymphotactin on chickens vaccinated with the DNA vaccine. Lymphotactin gene, when given simultaneously with the pcDNA3-1E vaccine, significantly protected chickens against body weight loss induced by *E. acervulina* infection. Parasite replication was also significantly reduced in chickens immunized with the pcDNA3-1E vaccine and co-injected with IL-8, lymphotactin, IFN-γ, IL-15, TGF-β4, or IL-1β gene as compared to chickens given the pcDNA3-1E vaccine alone. These results provide the first direct evidence that chicken cytokines exert a protective effect against Eimeria and provide a rational basis for the use of this cytokine as a vaccine

adjuvant against coccidiosis. The effects of dietary supplements such as vitamin A and probiotics have been studied in coccidiosis. Overall, dietary vitamin A levels can affect gut immunity in broiler chickens and its deficiency can cause immunosuppression at those sites resulting in increased susceptibility to coccidiosis. Probiotic supplementation of the intestinal microflora has been shown to enhance gut defensive mechanisms in poultry. We have demonstrated that a *Lactobacillus*-based probiotic stimulated the local immune system of broiler chickens and improved resistance to coccidia. Flow cytometric analysis of duodenum IEL showed that chickens fed the probiotic-supplemented diet had a significant increase in IEL T lymphocyte subpopulations and indicated that the probiotic enhanced local immune defenses in the intestinal tract of broiler chickens resulting in better resistance to *E. acervulina.* A greater understanding of the mechanisms of enhancement of intestinal immunity using various dietary supplements would improve the effectiveness of using these feed additives for disease control. Short oligodeoxynucleotides (ODN) containing unmethylated CpG motifs have been shown to be effective immunoprotective agents in mammalian models, by inducing both innate and adaptive immune responses. We have recently identified CpG sequences that activate chicken innate immunity and enhance protective immune response against *Salmonella* and coccidia. In multiple trials, CpG ODN provided great potential as vaccine adjuvants in chickens and enhanced disease resistance against *E. acervulina* infection. On the other hand, we found that feed supplementation with high levels of fat-soluble vitamin E (DL-alpha-tocopheryl acetate), a natural antioxidant, does not reduce pathology of mild or severe E. maxima infections in chickens. Plasma levels of Vitamin E declined by 60 to 70% during the acute phase of infection in a manner correlated with decreases in plasma carotenoids regardless of the level fed. It is postulated that ameliorative effects of vitamin E are not observed during E. maxima infections because the vitamin is prevented from access to infected tissues because of malabsorption and/or oxidation. Producers are thus cautioned against wasteful use of alpha-tocopheryl acetate as a dietary supplement to control avian coccidiosis. A number of plant products have immunostimulatory properties, and may be useful as feed additives to control coccidiosis. Echinacea purpurea can enhance immunity to challenge infection when fed to chicks immunized with suboptimal doses of live oocysts. Beta glucans from yeast and hops are currently being tested as immunostimulatory feed additives in various challenge protocols.

IMPACT:

1. The Poultry and Animal Health Industries have shown great interest in our vaccine research. A patent on recombinant coccidial vaccines (U.S. Patent 5,122,471) was licensed to Nippon Zeon, Inc. CRADAs (two) were established with Nippon Zeon to develop and field test a fowlpox virus vector system for coccidia vaccine (Jenkins/Lillehoj). Recombinant chicken antibody technology was the basis for the U.S. patent 6,451,984 and this technology was transferred to IDEXX Laboratories through a CRADA to develop novel control strategies against other economically important poultry pathogens. A CRADA with Intervet was established to produce and characterize new vaccine strains of *Eimeria* for incorporation into live vaccines (Allen) that are finding increased industrial acceptance.

2. Vital information on the drug and vaccine sensitivity trials were effectively communicated to the poultry producers and alerted them of emerging drug resistance. The information allows poultry producers to select the most efficacious drugs or vaccines to combat avian coccidiosis. CRADAs have been initiated with Townsends, Inc., Solutions Biosciences, Inc., and Schering-Plough to test the sensitivity of *Eimeria* strains isolated from broiler houses in various regions of the U.S. A highly specific and sensitive molecular technique based on PCR amplification of DNA coding for ribosomal RNA has been developed to rapidly determine the species composition of *Eimeria* oocysts in a broiler operation. In addition, the cooperative work with the commercial poultry operations provided new, often more virulent isolates of avian *Eimeria*, such that basic research is examining strains which are present in the field. These programs have led to the development of a novel and practical concept for enhancing poultry immunity using lymphokines. These reagents against chicken lymphokines have led to patent filing (Serial Number 09/534,002) and this technology led to the development of a CRADA with Novus International, Inc. to evaluate the efficacy of field trial data of a new coccidia vaccine delivery technology (Lillehoj) and a Trust agreement with Maine Biological Laboratories, Inc. to apply cytokines for enhanced immunity in poultry. In summary, these studies have set the stage for the future development of novel control strategies for poultry diseases.

3. The work on the chicken genome has provided the first description of an association of chicken genes with coccidiosis resistance. The chicken cDNA database will facilitate the identification of the nature of host genes controlling immunity, growth and disease resistance. These new bodies of information can be used by the poultry industry for marker-assisted selection of resistant breeding stocks without the need to conduct large numbers of experimental infections. The findings of an early impact of coccidia infection on the chick neuroendocrine system and muscle metabolism provide new foci for functional genetic selection of resistance and susceptibility coccidiosis disease resistance and development of new metabolic markers to assess the impact of coccidiosis on the important production parameters of growth and feed conversion.

4. Our research findings concerning novel vaccine strategies and the assessment of vaccine efficacy have been used by many poultry industry in developing new commercial coccidia vaccines. Through CRADA and Trust agreements, many technologies have been transferred to poultry industry worldwide.

TECHNOLOGY TRANSFER:

1. A contract with Intervet Inc. was established to isolate and prepare two *E. maxima* strains, and a strain of *E. acervulina* and *E. tennella* for use in live vaccines. *Eimeria acervulina* and *E. tenella* strains have been prepared and submitted. *Eimeria maxima* strains are currently undergoing purification and testing, including cross protection and drug sensitivity assessments.

2. Over 500 *E. tenella* cDNAs associated with oocyst development and >10,000 EST isolated from chicken cDNA library have been deposited onto public gene databanks. Deposited 14,374 chicken cDNA isolated from chicken intestine into NCBI Genbank (GenBank accession number:CD726833-CD740810 and CF074760-CF075157).

3. Developed PCR based methods to identify Eimeria species from field samples as part of a CRADA to develop control strategies against avian coccidiosis.

4. Developed MOU on "Application of Intellijject System to enhance poultry immunity" with AviTech Inc., Hebron, MD. And transferred technology on in ovo vaccination.

5. Contract with Phytosynthese Inc. was developed to test natural product preparations which are derived from a French company for anti-coccidial effectiveness.

6. Genes identified through screening of cDNA libraries which are important for parasite functions will be tested for potential vaccine activity.

7. A detailed knowledge of the genetic diversity within and between Eimeria species will be transferred to the poultry industry and will enable the implementation of precision agriculture technologies to apply treatments or combinations of treatments optimally suited to control the parasite variants in a particular locale.

8. The results of the identification of host genes which control coccidiosis and the development of DNA marker based strategy for coccidiosis resistance will be transferred to the poultry industry community.

9. New technologies enabling analysis and comparisons of multiple Eimeria species through genetic sequencing and analysis of gene products will allow the identification of DNA and or protein components that may be useful in the development of a multi-species subunit vaccine.

10. Vaccine technology, vaccine delivery strategies, and immunological technologies will be transferred to poultry vaccine companies for commercial applications. Poultry producers will be able to utilize commercially produced vaccines for the control of Eimeria in their flocks. Monoclonal antibodies, recombinant chicken cytokines, immunoassays for poultry health assessment and genetically engineered chicken antibodies will have immediate application to poultry studies by scientists in academia, industry and federal and state agencies.

11. Developed a highly reliable protocol for testing the drug sensitivity of field isolates of Eimeria obtained from commercial broiler houses. Developed a rigorous statistical evaluation method that provides information on the efficacy of specific anti-coccidial drugs against isolated field strains of Eimeria. This protocol was used to initiate two CRADAs with poultry (Townsends Inc) and pharmaceutical companies (Solutions Biosciences, Inc. and Schering-Plough).

Patents:

1. Kim J. K., Han, J. Y., Song, K. D., and Lillehoj, H.S. 2002. Recombinant ScFv antibodies specific to Eimeria spp. responsible for coccidiosis. Patent Korean (File no. 2001-52934) and Japan (File no. 2001-266333).

2. Lillehoj, H. S. and Nichols, M. 2002. Chicken monoclonal antibodies specific for coccidial antigens involved in invasion of host lymphocytes. US Patent 6,451,984

3. Allen, P. 2001. Applied a patent for the use of ground preparations of Echinacea purpurea feed additives to stimulate immunity elicited by live vaccination. Docket number 0217.01, Serial number 9/931,033 Date filed: 8/17/2001 Recordation date: 10/25/2001.

4. Lillehoj, H. 2000. Monoclonal antibodies detecting chicken IFN-gamma and development of in vitro assay. Serial Number 09/534,002, Document Number 0165.99. Patent filed 3/24/00.

5. Yasuda, et al., 2000. Novel DNA encoding *Eimeria* glyceroaldehyde-3-phosphate dehydrogenase and uses thereof. Filed by Nippon Zeon Inc.

6. Lillehoj, H.S., Dalloul, R. Nichols, M., and Chung, K. S., "Immunopotentiating effect of Formitella fraxinea mushroom lectin on poultry immunity to coccidiosis. Docket No. 0161.03.

7. Heckert, R. A., Xie, H. and Lillehoj, H. S., " Immunomodulation of poultry by oligonucleotides". Filed by the Office of Technology Commercialization, University of Maryland. November, 2003.

Cooperative Research and Development Agreements (CRADA)

58-1265-9-058 (Trust Fund Agreement with Roche Vitamins)
Effect of dietary vitamin E supplementation on chickens infected with *Eimeria maxima*.
Investigator, P.C. Allen Funding: $23,000
Start: 09/01/1999 Termination: 08/31/2001

58-1265-2-F091 (Trust Fund Agreement with Phytosynthese Inc.)
Anticoccidial Effects of Plant-Derived Products
Investigator: P.C. Allen Funding: $6,000
Start: 02/28/ 2002 Termination: 02/28/2003

1265-31320-070-13T (Trust Fund Agreement with Intervet)
Production of vaccine strains suitable for incorporation in a live vaccine for avian coccidiosis.
Investigator: P.C. Allen Funding: $59,000
Start1: 01/01/2001 Termination: 01/01/2004

333-1265-140 (ISTC Project with Former Soviet Union Countries)
ISCOM delivery of recombinant coccidia (Eimeria) antigens**.**
Investigator: R. Fetterer Funding: $ 40,000
Start: 01/01/01 Termination: 01/01/04
*Renewed Start: 09/01/2003 to 09/30/2006 with new funding $ 40,000.

1265-32000-061-15T (CRADA with Nippon Zeon, Japan)
Development of a coccidiosis vaccine in recombinant fowlpox virus and HVT
Investigator: Mark C. Jenkins Funding: $ 100,000
Start: 04/01/1999 Termination: 03/31/2003
58-1265-2056 (Trust Fund Cooperative Agreement with Townsends, Inc)
Testing of drug and vaccine sensitivity of Eimeria field isolates
Investigator: Mark C. Jenkins Funding: $ 15,000
Start: 08/31/2002 Termination: 09/01/2007

58-1265-2019 (Trust Fund Cooperative Agreement with Solutions Biosciences, Inc.)
Testing of drug and vaccine sensitivity of Eimeria field isolates
Investigator: Mark C. Jenkins Funding: $ 25,000
Start: 04/15/2002 Termination: 04/14/2006

1265-32000-050-08T (Trust Agreement with IDEXX Corp.)
Development of chicken hybridomas and other immunological reagents for chickens
Investigator: Hyun Lillehoj Funding: $ 50,000
Start: 01/01/1997 Termination: 09/01/2002

1265-32000-050-10T (CRADA with Nippon Zeon Corp.)
Development of fowlpox virus vector system for coccidia and cytokine gene delivery to enhance poultry immunity
Investigator: Hyun Lillehoj Funding: $ 422, 600
Start: 03/01/2001 Termination: 12/06/2002

1265-20-00-11237 (Trust Agreement with Novus International Inc.)
Development of immunological reagents for the avian system
Investigator: Hyun Lillehoj Funding: $ 12,000
Start: 02/01/1997 Termination: 01/01/2000

1265-32000-050-12T (CRADA with Novus International Inc.)
Development of mucosal immunization strategy against coccidiosis
Investigator: Hyun Lillehoj Funding: $ 150,367
Start: 07/01/1998 Termination: 06/01/2000

1265-32000-050-14T 15R (CSREES National Research Initiative Grant)
Interferon-gamma-mediated immune enhancement in avian coccidiosis
Investigator: Hyun Lillehoj Funding: $ 96,000
Start: 09/01/1998 Termination: 08/31/2003

1265-32000-050-13T (Trust Agreement with Maine Biological Laboratory)
Application of cytokines and immunological reagents for enhanced immunity in chickens
Investigator: Hyun Lillehoj Funding: $ 50,000
Start: 02/01/1998 Termination: 01/31/2003

1265-31320-070-01R (Fund for Rural America Grant)
DNA marker technology in commercial broiler selection programs
Investigator: Hyun Lillehoj Funding: $ 320,000
Start: 02/01/1998 Termination: 02/01/2001
1265-32000-050-23R (Reimbursable Agreement with University of Maryland)
Development of in vitro cytokine assays for studying mucosal immunity in chickens
Investigator: Hyun Lillehoj Funding: $ 148,589
Start: 09/01/2000 Termination: 08/31/2005

1265-31320-014-04T (Trust Agreement with Ghen Immunology Institute)
Development of novel immunological control strategy using hyperimmune egg antibodies to prevent against coccidiosis
Investigator: Hyun Lillehoj Funding: $ 20,000
Start: 03/01/2001 Termination: 02/28/2006

1265-31320-014-03T (Trust Agreement with Avicore Biotechnology Institute)
Application of functional genomics to develop novel control strategies against poultry diseases
Investigator: Hyun Lillehoj Funding: $ 240,000
Start: 04/01/2001 Termination: 03/30/2006

1265-31320-070-15R (CSREES National Research Initiative Grant)
Mapping quantitative trait associated with disease resistance in commercial broiler chickens
Investigator: Hyun Lillehoj Funding: $ 200,000
Start: 01/01/2003 Termination: 12/30/2005

1265-31320-070- (CRADA Agreement with Zeon Corp.)
DNA vaccination strategy through embryonic vaccination
Investigator: Hyun Lillehoj Funding: $ 20,000
Start: 04/01/2003 Termination: 03/30/2008

1265-31320-070-T (Trust Agreement with Novus International Inc.)
Assessment of coccidia immune response using interferon-gamma assay
Investigator: Hyun Lillehoj Funding: $ 10,000
Start: 04/01/2002 Termination: 03/30/2007

Agreement No. 58-1265-4-F060 (Cooperative Research Project Between ARS and Rural Development Administration, South Korea)
Study on the mechanisms of immunomodulatory phytochemicals from fruits and vegetables in Republic of Korea.
Investigator: Hyun Lillehoj Funding: $ 72,000
Start: May, 1, 2004 Termination: April 30, 2007.

193-1265-154 (CRADA with Intervet Inc.) Production of vaccine strains suitable for incorporation in a live vaccine for avian coccidiosis.
Investigator : Patricia C. Allen; funding $59,000. Start 2/1/2002,; terminate 9/1/2005.

Other external agreement:
A material transfer agreement was established with Transfer Point, a manufacturer of yeast beta glucans to test effectiveness of the product as an anticoccidial feed additive.

A cooperative project with Gregory Siragusa, USDA, Russel Research Center, Athens GA, is under way to test anticoccidial activity of soluble beta glucans from hops.

A cooperative research project was undertaken with Bill Chobotar, Andrews University, to ultrastructurally locate the acetyl coA carboxylase complex in Eimeria sp.

PUBLICATIONS:

Allen:

Allen, P.C. 2000. Effects of treatments with cyclooxygenase inhibitors on chickens infected with *Eimeria acervulina*. Poultry Sci.79:1251-1258.

Allen, P.C. and. R.H. Fetterer, 2000. Effect of *Eimeria acervulina* infections on plasma L-arginine.Poultry Sci. 79:1414-1417.

Allen, P.C., H.D. Danforth, , and, P.A. Stitt. 2000. Effects of nutritionally balanced and stabilized flax-meal based diets on *Eimeria tenella* infections in chickens. Poultry Sci. 79:1506-1509.

Fetterer, R.H. and. P.C. Allen, 2000. *Eimeria acervulina* infection elevated plasma and muscle 3-methythistidine in chickens. J. Parasitology 86:783-791.

Zhu, J., H. Lillehoj, P.C. Allen, C.-H. Yun, D. Pollock, M. Sadjadi and M. Emara, 2000. Analysis of disease resistance associated parameters in broiler chickens challenged with Eimeria maxima. Poultry Sci. 79:619-625.

Fetterer, R.H., and P.C. Allen , 2001. *Eimeria tenella* infections in chickens: Effect on plasma and muscle 3-methylhistidine. Poultry Sci. 80:1549-1553.

Allen, P.C. and Fetterer, R.H. 2002. Interaction of dietary vitamin E with *Eimeria maxima* infections in chickens. Poultry Science 81: 41-48.

Allen, P.C. and Fetterer, R.H. 2002. Recent advances in biology and immunobiology of Eimeria species and in diagnosis and control of infection with these coccidian parasites of poultry. Clinical Microbiology Reviews 15:58-65.

Allen, P.C., and R.H. Fetterer. 2002. Effects of dietary vitamin E on chickens infected with *Eimeria maxima*: Observations over time of primary infection. Avian Diseases. 46:839-846

Allen, P.C. 2003. Dietary supplementation with Echinacea and development of immunity to challenge infection with coccidia. Parasitol Research. Sep;91(1):74-8.

Fetterer RH, Augustine PC, Allen PC, Barfield RC**.** 2003. The effect of dietary betaine on intestinal and plasma levels of betaine in uninfected and coccidia-infected broiler chicks. Parasitol Res. 2003 Jul;90(4):343-8

Zhu, J.J., H.S. Lillehoj, P.C. Allen, C.P.Van Tassel, T.S. Sonstegard, H.H. Cheng, D. Pollock, M. Sadjadi, W. Min, and M.G. Emara., 2003. Mapping Quantitative trait loci associated with resistance to coccidiosis and growth. Poultry Sci. 82:9-16.

Allen, P.C., H.D. Danforth and B.T. Vinyard 2004. Development of a protective index to rank effectiveness of multiple treatments within an experiment: Application to a cross protection study of several strains of Eimeria maxima and a live vaccine. Avian Diseases, in press.

Allen, P.C., 2004. Oxidative stress during avian coccidiosis. Poultry Sci. in press

Fetterer:

Allen, P.C., Fetterer, R.H. 2000. Effect of Eimeria acervulina infections on plasma l-arginine. Poultry Science.. v. 79. p. 1414-1417.

Fetterer, R.H., Allen, P.C. 2000. Eimeria acervulina elevates plasma and Muscle 3-methylhistindine levels in chickens. Journal of Parasitology. v 86. p. 783-781.

Fetterer,R.H.,Allen,P.C. 2001. Eimeria tenella infection in chickens: effect on plasma and muscle 3-methyl histidine. Poultry Science. v.80. p.1459-1563.

Fetterer,R.H.,Augustine,P.C. 2001. Turkey coccidiosis results in elevation of muscle and plasma 3-methyl histidine. Poultry Science. v.45. p.783-840.

Allen,P.C.,Fetterer,R.H. 2002. Interaction of dietary vitamin E with Eimeria maxima infections in chickens. Poultry Sciences. . v.81. p.41-48.

Allen,P.C.,Fetterer,R.H. 2002. Recent advances in biology and immunobiology of Eimeria species and in diagnosis and control of infecion with these coccidian parasites of poultry. Clinical Microbiology Review. v.15. p.58-65.

Min, W., Lillehoj, H.S. and Fetterer, R.H. 2002. Identification of alternatively spliced isoform of the common cytokine receptor gamma chain in chickens. Biochemical Biophysical Research Communications. v299. 321-27

Fetterer, R.H., P.C. Augustine, P.C. Allen and Barfield, R.C. 2003. The effect of Dietary Betaine on intestinal and plasma levels of betaine in uninfected and coccidian-infected broiler chicks. Parasitology Research 2003 v. 90. p. 343-348

Fetterer, R.H. and Barfield, R.C. 2003. Characterization of a developmentally regulated oocyst protein from Eimeria tenella. Journal of Parastiology 2003. v. 89. 553-564.

Fetterer, R. H. K. Miska, M. Jenkins and R. C. Barfield. 2004. A conserved 19 kDa Eimeria antigen is a profiling-like protein. Journal of Parasitology 2004 (In press).

Jenkins:

Lillehoj, H.S., Choi, K.D., Jenkins, M.C., Vakharia, V.N., Song, K.D., Han, J.Y., and Lillehoj, E.P. 2000. A recombinant Eimeria protein inducing interferon-gamma production: comparison of different gene expression systems and immunization strategies for vaccination against coccidiosis. Avian Dis. 44:379-389.

Jenkins, M.C.2001 Advances and prospects for subunit vaccines against protozoa of veterinary importance. Vet. Parasitol. 101:291-301.

Fetterer, R. H. K. Miska, M. Jenkins and R. C. Barfield. 2004. A conserved 19 kDa Eimeria antigen is a profiling-like protein. Journal of Parasitology (In press).

Jenkins, M.C**.**, Allen, P.C., Fetterer, R.H., Lillehoj, H.S., Miska, K.B. 2004 Are there immunovariants of coccidia that can affect vaccine efficacy? (The clues may not always be obvious). Watt Poultry USA.

Jenkins, M.C. 2004 Control of avian coccidiosis: Drugs and vaccines. Feed Info News Scientific Reviews. Available from URL: http://www.feedinfo.com.

Lillehoj:

Lillehoj, H. S. 2000. Mucosal Immune response to coccidiosis. In “Proc. XXI World Poultry Congress”. S1.10. (Published on CD).

Lillehoj, H. S. 2000. Review on vaccine development against enteric parasites *Eimeria* and *Cryptosporidium*. Jap. Poul. Sci. 37: 117-141.

Lillehoj, H. S. 2000. Subcutaneous injection with IL-2 gene enhances cell-mediated immunity. In "Proc World Poultry Congress," P17.14. (Published on CD).

Lillehoj, H. S., and Lillehoj, E. P. 2000. Avian coccidiosis. A review of acquired intestinal immunity and vaccination strategies. Avian Dis. 44:408-425.

Lillehoj, E. P., Yun, C. H. and Lillehoj, H. S. 2000. Vaccine for Avian enteropathogens *Eimeria, Cryptosporidium* and *Salmonella*. Animal Health Research Reviews. 1:47-65.

Lillehoj, H. S., Jenkins, M.C., Vakharia, V. 2000. Eimeria protein inducing interferon-γ production. Comparison of different gene expression systems and immunization strategies for vaccination against coccidiosis. In "Proc. World Poultry Congress," P17.13. (Published on CD).

Lillehoj, H. S., Choi, K. D., Jenkins, M. C., Vakharia, V. N., Song, K. D., Han, J. Y. and Lillehoj, E. P. 2000. A recombinant *Eimeria* protein inducing interferon-γ production. Comparison of different gene expression systems and immunization strategies for vaccination against coccidiosis. Avian Dis. 44:379-389.

Yun C. H., and Lillehoj, H. S. 2000. Intestinal immune responses to coccidiosis. Dev. Comp. Immunol. 24:303-324.

Choi, K. D., and Lillehoj, H. S. 2000. Effect of chicken IL-2 on T-cell growth and function. Increased T cell response following DNA immunization. Vet Immunol Immunopathol. 73:309-312.

Yun, C. H., Lillehoj, H. S. and Choi, K. D. 2000. Chicken IFN-γ monoclonal antibodies and their application in enzyme-linked immunosorbent assay. Vet. Immunol. Immunopathol. 73:297-308.

Yun, C. H., Lillehoj, H. S. and Choi, K. D. 2000. *Eimeria tenella* infection induces local IFN-γ production and intestinal lymphocyte subpopulation changes. Infect. Immunity. 68: 1282-1288.

Yun, C. H., Lillehoj, H. S., Zhu, J. and Min, W.G. 2000. Kinetic differences in intestinal and systemic interferon-γ and antigen-specific antibodies in chickens infected with *Eimeria maxima*. Avian Dis. 44:305-312.

Zhu, J., Lillehoj, H. S., Allen, P., Yun, C. H., Pollock, D. and Emara, M. 2000. Analysis of disease resistance associated parameters in broiler chickens challenged with *Eimeria maxima*. Poul. Sci. 79:619-625.

Song, K. D., Lillehoj, H. S. Choi, K. D., Parcells, M. S., Huynh, J. T., Yun, C. H. and J.Y. Han. 2000. A DNA vaccine encoding a conserved *Eimeria* protein induces protective immunity against live *Eimeria acervulina* challenge. Vaccine. 19:243-252.

Sasai, K., Aita. M., Lillehoj, H. S., Miyamoto, T., Fukata, T and E. Baba. 2000. Dynamics of lymphocyte subpopulation changes in the cecal tonsils of chickens infected with *Salmonella enteritidis*. Vet. Microbiol. 74:345-351.

Kliger, C. A., Gehad, A. E., Hulet, R. M., Roush, W. B., Lillehoj, H. S., and Mashaly, M. M. 2000. Effect of melatonin and photoperiod on broiler immune system. Poultry Sci. 79:18-25.

Lillehoj, H. S., Yun, C. H. and Nichols, M. 2000. Monoclonal antibodies detecting chicken IFN-gamma and development of in vitro assay. Patent 09/534,002.

Lillehoj, H. S. 2001. Application of DNA marker Technology to the development of coccidiosis control strategy. In “Poultry Biotechnology and its Applications”, Proceeding of Korean Society of Poultry Science Symposium. pp1-17, Suwon, Korea.

Lillehoj, H. S.; Min, W.; Choi, K. D.; Babu, U. S.; Burnside, J.; Miyamoto, T.; Rosenthal, B. M. and Lillehoj, E. P. 2001. Molecular, cellular, and functional characterization of chicken cytokines homologous to mammalian IL-15 and IL-2. Vet. Immunol. Immunopath. 82: 229-244.

Lillehoj, H. S., Min, W. G., Choi, K. D., Babu, U., Burnside, J., Miyamoto, T. and E. P. Lillehoj. 2001. Functional Characterization of Chicken Cytokines Homologous to Mammalian IL-15 and IL-2. In "Current Progress on Avian Immunology Research," K. A. Schat ed, American Association of Avian Pathologists, pp8-13, Kennett Square, PA.

Lillehoj, H. S., Zhu, J and Min. W.G. 2001. Application of DNA marker Technology and functional genomics to the development of coccidiosis control strategy. Korean J. Poultry Sci 28:107-123.

Guangxing, Li, Lillehoj, Erik, and Lillehoj, H. S. 2002. Interleukin-2 production in SC and TK chickens infected with *Eimeria tenella*. Avian Diseases. 46:2-9.

Guangxing Li, Lillehoj, Hyun S. and Min, Wongi. 2001. Production and Characterization of Monoclonal Antibodies Detecting the Chicken Interleukin-15 Receptor Alpha Chain. Vet. Immunol. Immunopathol. 82: 215-227.

Gehad A. E., Lillehoj, H. S. and Mashaly, M. 2001. The role of lymphocytes and hormones during the development of the delayed hypersensitivity response in immature male chickens. In "Current Progress on Avian Immunology Research," K. A. Schat, ed., American association of Avian Pathologists, pp308-314, Kennett Square, PA.

Kim, J. K., Lillehoj, H. S. Min, W. G., Han, J. Y., Sasai, K. , Sohn, E. J., and Lillehoj, E. P. 2001. Recombinant Single Chain Fragment Variable Region Antibody Constructed from Chicken Monoclonal Antibody Detects Apical Complex Protein of *Eimeria acervulina*. In "Current Progress on Avian Immunology Research," K. A. Schat, ed., American Association of Avian Pathologists, pp 64-70, Kennett Square, PA.

Min, W. G., Lillehoj, H. S., Burnside, J., Weining, K. C., Staeheli, P. and Nichols, M B. 2001. Effect of IL-1, IL-8, IL-15, IL-2, IFN-α, IFN-γ, Lymphotactin and TGF-β4 on DNA vaccination against *Eimeria acervulina*. In "Current Progress on Avian Immunology Research," K. A. Schat, ed, American Association of Avian Pathologists, pp352-356, Kennett Square, PA.

Zhu, J., Lillehoj, H. S., Cheng, H. H., Pollock, D., Sadjadi M. and Emara, M. 2001. Screening for highly heterozygous chickens in outbred commercial broiler lines to increase detection power for mapping quantitative trait loci. Poul Sci. 80:6-21.

Miyamoto, T., Lillehoj, H. S., Sohn, E. J. and W. Min. 2001. Production and characterization of monoclonal antibodies detecting chicken interleukin-2 and the development of an antigen capture enzyme-linked immunosorbent assay. Vet. Immunol. Immunopathol. 80:245-257.

Min, W. G., Lillehoj, H. S., Burnside, J., Kim, H. B. and E. Lillehoj. 2001. Northern blot analysis of concanavalin A-induced expression of chicken gene homologous to the mammalian IL-2 receptor chain. In "Current Progress on Avian Immunology Research," Ed. K. A. Schat, American Association of Avian Pathologists, pp346-351, Kennett Square, PA.

Min, W., Kim, J. K., Lillehoj, H. S., Sohn, E. J., Han, J. Y., Song, K. D. and Lillehoj E. 2001. Characterization of recombinant scFv antibody reactive with an apical antigen of Eimeria acervulina. Biotechnology Letters 23:949-955.
Kim, J. K., Min, W., Lillehoj, H. S., Kim, S. W., Sohn, E. J., Song, K. D. and Han, J. Y. 2001. Generation and characterization of recombinant scFv antibodies detecting Eimeria acervulina surface antigens. Hybridoma 20:175-181.

Miyamoto, T., Min, Wongi, and Lillehoj, H. S. 2002. Lymphocyte proliferation response during *Eimeria tenella* infection assessed by a new, reliable, non-radioactive colorimetric assay. Avian Diseases 46:10-16.

Miyamoto, T., Min, W. and Lillehoj, H. 2002. Kinetics of interleukin-2 production in chickens infected with *Eimeria tenella*. Comparative Immunology, Microbiology and Infectious Diseases, 25:149-158.

Song, K. D., Han, J. Y., Min, Wongi, Lillehoj, H. S., Kim, S. and Kim, J..2001. Molecular cloning and characterization of cDNA encoding immunoglobulin heavy and light chain variable regions from four chicken monoclonal antibodies specific to surface antigens of intestinal parasites, *Eimeria acervulina*. The Korean J. Microbiology. 39(1): 49-55.

Hwang, K. C., Song, K. D., Kim, T. H., Lee, H. K., Sohn, S. H., Lillehoj, H. S. and Han, J. Y. 2001. Genetic linkage mapping of RAPD markers segregating in Korean Ogol chickens-White Leghorn backcross population. Asian-Aust. J. Anim. Sci. 14:302-306.

Kim J. K., Han, J. Y., Song, K. D., and Lillehoj, H.S. 2002. Recombinant ScFv antibodies specific to *Eimeria* spp. responsible for coccidiosis. Patent Korean (File no. 2001-52934) and Japan (File no. 2001-266333).

Lillehoj, H. S. 2002. Beyond genomics revolution: Emergence of agricultural biotechnology. In "Proceeding of Future Perspectives in Animal Biotechnology," pp 5-21, Seoul National University, College of Animal Science and Technology, Suwon, Korea.

Lillehoj, H. S. 2002. Gut immune responses and intervention strategies against Eimeria and Salmonella. In "Proceeding of International Seminars on Avian Pathology and Production," Pp 91-131, Chile, Santiago.

Lillehoj, H. S. 2002. Coccidiosis: This poultry disease's impact is anything but paltry. Healthy Animals, On-line quarterly USDA-ARS Newsletter. February Issue 10.

Yamage, M. and Lillehoj, H. S. 2002. Immunity to protozoan parasites in poultry. In "Modern Concepts of Immunology in Veterinary Medicine-Poultry Immunology," In Advances in Medical and Veterinary Virology, Immunology and Epidemiology, T. Matthew. Thajema ed., Thajema Press, pp183-242.

Min, Wongi, Lillehoj, H. S., Burnside, J. Weining, K. C., Staeheli, P. and Nichols, M. 2002. Adjuvant effects of IL-1beta, IL-8, IL-15, IFN-alpha, IFN-gamma, TGF-beta and Lymphotactin on DNA vaccination against Eimeria acervulina. Vaccine. 20:267-274.

Gehad, A. E., Lillehoj, H. S., Hendrick III, G.L. and Mashaly, M.. 2002. I. The role of cytokines and hormones in the initiation of humoral immunity using T-independent and T-dependent antigens. Develop. Comp. Immunol. 26:751-759.

Gehad, A. E., Lillehoj, H. S., Hendrick, G. L. and Mashaly, M. 2002. II. The effects of T-independent and T-dependent antigens on the distribution of lymphocyte populations. Devel. Comp. Immunol. 26:761-771.

Min, W., Lillehoj, H. S., Guangxing, L., Sohn, E. J., and Miyamoto, T. 2002. Development and characterization of monoclonal antibodies to chicken interleukin-15. Vet. Immunol. Immunopathol. 88:49-56.

Emara, M. G., Lapierre, R.L., Greene, G. M., Knieriem, M., Rosenberger, J. K., Kim, C. and Lillehoj, H.S. 2002. Phenotypic variation among three broiler pure lines for Marek's Disease, coccidiosis and antibody response to sheep red blood cells. Poultry Science 81:642-648.

Olah, I., Gumati, K. H., Nagy, N., Magyar, A., Kaspers, B. and Lillehoj, H. 2002. Diverse expression of the K-1 antigen by cortico-medullary and reticular epithelial cells of the bursa of Fabricius in chicken and guinea fowl. Devel. Comp Immunology 26:481-488.

Emara, M. G., Kim, H, Lapierre, R. R., Lakshmanan, N., Pollock, D. L., Sadjadi, M. and Lillehoj, H. S. 2002. Genetic diversity at the major histocompatibility complex (B) and microsatellite loci in three commercial broiler pure lines. Poul. Sci. 81:1609-1617.

Babu, U., Scott, M., Myers, M. J., Okamura, M., Gaines, D., Raybourne, R., Yancy, H. F., Lillehoj, H., and Heckert, R. 2002. Effects of live attenuated and killed Salmonella vaccine on T-lymphocyte mediated immunity in laying hens. Vet. Immunol. Immunopathol. 6729:1-6.

Min, Wongi, Lillehoj, H. and Fetterer, R. 2002. Identification of an alternatively spliced transcript isoform of the common cytokine receptor γ chain. Biochem. Biophys. Res. Com. 299: 321-327.

Min#, W., and Lillehoj, H. S. 2002. Sequence and functional properties of chicken interleukin-17. J. Interferon & Cytokine Res. 22:1125-1130.

Lillehoj, H. S., and Min. W. G. 2002. Gallus gallus mRNA for interleukin-16. Genbank. Accession Number. AJ508678.

Lillehoj, H. S., and Min, W. G. 2002. Gallus gallus mRNA for putative common cytokine receptor gamma chain β. Genbank. Accession Number. AJ419896.

Lillehoj, H. S., and Min, W. G. 2002. Gallus gallus mRNA for putative common cytokine receptor gamma chain α. Genbank. Accession Number. AJ419898.

Marie-Helene Pinard-van der Laan, Lillehoj, H.S. and Zhu, J. 2003. Disease resistance and transmission. Genetic resistance and transmission of avian parasites. In "Poultry Genetics, Breeding and Biotechnology", W. M. Muir, and S. E. Aggrey eds. CABI Publishing, UK.

Zhu J., Lillehoj, H. S., Allen, P.C., Min, W., Van Tassell, C., Sonstegard, T. S., Cheng, H. H., Pollock, D. L., Sadjadi, M. and Emara, M. 2003. Mapping quantitative trait loci associated with resistance to coccidiosis and growth. Poultry Science:82:9-16.

Dalloul*, R. A., Lillehoj, H. S. and Doerr, J. A. 2003. Effect of vitamin A deficiency on local and systemic immune responses of broiler chickens. Poultry Science 81: 1509-1515

Zhou*, Huaijun, Lillehoj, H. S and Lamont, S. 2003. Association of chicken interferon-γ genotype and protein level with antibody response kinetics. Avian Diseases: 46:869-876.

Dalloul*, R. A., Lillehoj, H. S., Shellem, T. A. and Doerr, J. A. 2003. Enhanced mucosal immunity against Eimeria acervulina in broilers fed a Lactobacillus-based probiotic. Poul. Sci. 82:62-66.

Babu, U., Scott, M., Myers, M. J., Okamura, M., Gaines, D., Raybourne, R., Yancy, H. F., Lillehoj, H., and Heckert, R. 2002. Effects of live attenuated and killed Salmonella vaccine on T-lymphocyte mediated immunity in laying hens. Vet. Immunol. Immunopathol. 6729:1-6.

Xie, H., Raybourne, R. B., Babu, U., Lillehoj, H. and Heckert, R. A. 2003. Effects of CPG on IL-6 and nitric oxide production, surface antigen expression, phagocytosis, proliferation and apoptosis of chicken macrophage cell line HD11 cells. Dev. Comp. Immunol. 27(9):823-834.

Okamura, M., Lillehoj, H.S., Raybourne, R. B., Babu, U. and Heckert, R. 2003. Antigen-specific lymphocyte proliferation, Interleukin-6 (IL-6) and IL-2 production in chickens immunized with outer membrane protein of Salmonella enteritidis. Avian Diseases. 47:1331-1338.

Min, Wongi, Lillehoj, H.S., Kim, Sungwon, Zhu, J. J., Beard, H., Alkharouf, N. and Matthews, B. F. 2003. Profiling local gene expression changes associated with Eimeria maxima and Eimeria acervulina using cDNA microarray. Appl Microbiol Biotechnol 62:392-399.

Lillehoj, H. and Okamura, M. 2003. Host immunity and vaccine Development to coccidia and *Salmonella* infections in chickens. J. Poul. Sci 40:151-193.

Withanage, G.S., Sasai, K., Fukata, T., Miyamoto, T., Lillehoj, H.S., and Baba, E. 2003. Increased lymphocyte subpopulations and macrophages in the ovaries and oviducts of laying hens infected with *Salmonell*a enterica serovar *Enteritidis.* Avian Pathol 32:583-590.

Constantinoiu, C.C., Lillehoj, H. S., Matsubayashi, M., Hoshoda1, Y., Tani1, H., Matsuda4, H., Sasai.1*, K., and Baba1, E. 2003. Chicken Monoclonal antibodies to stage-specific and cross-reactive antigens of *Eimeria acervulina*. Vet. Parasitol. 118:29-35.

Lillehoj H. S., Dalloul, R. and Min, W. 2003.Enhancement of intestinal immunity to coccidiosis using cytokines, oligodinucleotides and probiotics. 2003. World Poultry: Special Edition. pp . 18-23.

Min, W., and Lillehoj, H.S. 2004. Identification and characterization of chicken interleukin-16 cDNA. Dev. Comp. Immunol. 28:153-162.

Dalloul, R. A. Lillehoj, H. S., Shellem, T. A., and Doerr, J. R. 2004. Intestinal Immunomodulation by Vitamin A Deficiency and Lactobacillus-based Probiotic in Eimeria acervulina-infected Broiler Chickens. Avian Dis. In press.

Okamura, M., H. S. Lillehoj*2, R. B. Raybourne†, U. S. Babu† and R. A. Heckert‡ 2004. Cell-mediated Immune Responses to a killed Salmonella enteritidis Vaccine: Comp. Immunol Microbiol Infect Dis. in press.

Lillehoj, H. S., Min, W.G., and Dalloul, R. A. 2004. Recent Progress on the Cytokine Regulation of Intestinal Immune Responses to Eimeria. Poul. Science. In press.

Lillehoj, H.S. and Guangxing L.2004. Nitric oxide production by macrophages stimulated with sporozoites, lipopolysaccharide, or IFN-γ and its dynamic changes in SC and TK strains of chickens infected with Eimeria tenella. .In press.

Jenkins, M., Allen, P., Fetterer, R., Lillehoj, H, Miska, K. 2003. Are there immunovariants of coccidia that can affect vaccine efficacy? Watt Poultry USA.

Erf, G. F., Bersi, T. K. and Lillehoj, H. S. 2003. A role of interferon gamma in autoimmune vitiligo of Smyth line chickens. FEMS Immunology and Medical Microbiology. In press.

Babu, U., Dalloul, R.A., Okamura, M., Lillehoj, H.S., Xie, H., Raybourne, R., Gaines, I., and Heckert, R. 2004. Salmonella enteritidis Clearance and Immune Responses in Chickens Following Salmonella Vaccination and Challenge. Veterinary Immunol Immunopathol. In press.

Miska, K., Fetterer, R, Min, W. and Lillehoj, H. Identification of a heat shock protein 90 gene in two *Eimeria* species: Expression and evolutionary analysis. Molecular and Biochemical Parasitology. In press.

Miska:

Canada N., Meireles C.S., Mezo, M., Warleta, M., Correia da Costa, J.M., Sreekumar, C., Hill, D.E., Miska K., Dubey, J.P. First isolation of *Neospora caninum* from an aborted bovine fetus in Spain. Journal of Parasitology, *in press*.

Dubey J.P., Sreekumar C., Miska K.B, Hill D.E., Vianna M.C, Lindsay D.S. Molecular and biological characterization of *Hammondia heydorni* -like oocysts from a dog fed hearts from naturally-infected white-tailed deer (*Odocoileus virginianus*). Journal of Parasitology, *in press*.

Miska, KB Fetterer RH, Barfield RC. Analysis of transcripts expressed by *Eimeria tenella* oocysts using subtractive hybridization methods. Journal of Parasitology, *in press*.

R.H. Fetterer, K.B. Miska, M.C. Jenkins, R.C. Barfield. A conserved 19 kDa *Eimeria tenella* antigen is a profilin-like protein. Journal of Parasitology*, in press*.

Sreekumar, C., Hill, D.E., Miska, K.B., Rosenthal, B.M., Vianna, M.C.B., Venturini, L., Basso, W., Gennari, S.M., Lindsay, D.S., Dubey, J.P. *Hammondia heydorni*: evidence of genetic diversity among isolates from dogs. Experimental Parasitology*, in press*

Miska K.B., Min, W., Lillehoj H.S., Fetterer, R.H. Identification of a Heat shock protein 90 gene in two *Eimeria* species: expression and evolutionary analysis. Submitted to Journal of Parasitology.

Rodrigues, A.A.R., Aguiar, D.M., Sreekumar, C., Hill, D.E., Miska, K.B., Vianna, M.C.B, Gennari, S.M., Dubey, J.P. Shedding of *Neospora caninum* oocysts by dogs fed tissues from naturally infected water buffaloes (*Bubalus bubalis*) from Brazil. Submitted to Veterinary Parasitology.

Dubey, J. P., Sreekumar, C., Knickman, E., Miska, K.B., Vianna, M.C.B., Kwok, O.C.H., Hill, D. E., Jenkins, M.C., Lindsay, D.S., Greene, C.E. Clinical, biologic, morphologic, and molecular characterisation of *Neospora caninum* infections in littermate dogs. Submitted to International Journal of Parasitology.

Animal Parasitic Diseases Laboratory (APDL)

Project Title: Strategies to Control Swine Parasites Affecting Food Safety
CRIS Number: 1265-32000-064-00D
Scientists: Urban Jr, J., Lunney, J., Zarlenga, D.
Location: Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory
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PROJECT OBJECTIVES:
Swine have two prominent foodborne parasitic disease agents: the muscle worm, Trichinella spiralis, and the tissue protozoan parasite, Toxoplasma gondii. Infective eggs from the large roundworm, Ascaris suum, represent an emerging disease where agricultural use of pig manure contaminated with parasite eggs may increase human infection. Outbreaks of human disease from swine products negatively reflect on pork as a safe source of nutritious edible tissue and lowers export potential. Molecular tests to evaluate parasite infections in meat products need to be improved so that they are very sensitive and accurately reflect the true source of the infection.

Proper maintenance of herd health precludes infection but requires a large capital investment and breaches in procedure can readily result in new infections. No drugs are available to adequately cure tissue parasites, and the cost of development of new drugs by the pharmaceutical industry is prohibitive and unpredictable. Further, secondary bacteria disease is associated with worm infection in livestock and bacteria like Campylobacter is a major cause of diarrhea in humans. A long-term preventative control strategy is to vaccinate against swine parasites. Cooperative efforts between university, industry and government laboratories is directed at molecular regulators of immunity, receptor biology, innate protective mechanisms, and antibody responses targeted at the intestinal immune system of pigs. The basic nature of this work will also provide information for the immunological control of microbial and viral infection in swine.

Consumers are now requesting safer food products, thus encouraging producers to raise animals without antibiotics. Thus, producers have had to reshape the methods that they employ for raising disease-free pigs. This will require advanced knowledge of neonatal swine immunity and molecular tests for activators of the swine immune system such as recombinant cytokines [interleukin-12 (IL-12) and IL-18]. Description of effects of these cytokines and of synthetic oligonucleotides (CpG) on swine immunity, and their use to enhance neonatal swine immunity and to stimulate appropriate protective mechanisms against food borne pathogens, will have commercial value. Vaccines incorporating these activators would complement existing control procedures and provide the benefits of long-term protection, cost efficiency and reduced drug residues in the food chain. Studies of toxoplasmosis have shown that strong protective immunity can be induced in pigs by irradiated oocysts and attenuated strains, but that immunity is not complete and may result in low-level infection. Novel cytokine-based procedures that activate porcine immunity by increasing interferon-gamma (IFN-gamma) levels have the potential to enhance resistance to toxoplasmosis. The underdeveloped intestinal immune system of weaned-

pigs provides a model to test other prospective immune enhancing agents on development of resistance to infection in neonatal pigs.

Swine disease control is most important during the first month of a pig's life due to the immature immune system development in the newborn pig. Moreover there is rapid exposure of the newborn to infectious agents when it moves from the birthing (farrowing) unit into nursery facilities that house litters from numerous sows. We have asked whether administration of exogenous immune proteins, termed cytokines, enhances neonatal immunity and disease responses, and determines whether this will help protect young pigs from infections and stress-induced immune deficiencies. Our studies are aimed at determining the role of cytokines in the development of the neonatal immune system of swine. Descriptions of the mucosal immune system and the immunobiology of gastrointestinal parasite responses in neonatal and growing pigs will provide useful and largely unique information that will be available to scientists in other federal, academic and industry (producers, vaccine and pharmaceutical companies) laboratories.

Traditional genetic approaches have been very effectively used by the swine industry to enhance feed efficiency, pig meat production and reproductive traits. Recently, molecular methods have provided new genomic markers to actively select for specific traits such as the ESR gene for improved piglet number per litter. For pig health, inheritance of specific alleles within the swine leukocyte antigen (SLA) complex genes have been known to positively influence disease and vaccine responses. Broader pig health studies to determine whether defined immune markers could be correlated with specific resistance to disease are only now being approached. Results from these studies should provide producers with pigs that would require fewer antibiotic treatments and better disease resistance.

In the last year the disease target of this CRIS have been expanded to include Porcine Reproductive and Respiratory Syndrome (PRRS) and other respiratory diseases of swine. According to the National Pork Board (NPB), PRRS costs U.S. pork producers at least $600 million annually, and thus is the most economically significant disease facing the US industry today. PRRS affected 21.4% of all breeding herd operations and, importantly, 58.3% of operations with >500 sows (NAHMS 2000). From 16.6-50.7% of all grow/finish operations reported having PRRS within the previous 6 months. Losses are due not only to reduced reproduction capacity in gilts or sows, but to other aspects of production, poor growth and increased respiratory problems. Modified live virus vaccines are available for PRRS but protect only against homologous viral challenges. Because PRRSV is an RNA virus its genome changes and heterologous strains quickly arise. Thus new immune approaches are needed for prevention of PRRS using new biotherapeutics, improved vaccines and better adjuvants.

OVERALL PROJECT ACCOMPLISHMENTS:

Determination of the risk of foodborne transmission of Toxoplasma gondii in meat products requires a thorough epidemiological survey to allay consumer fears. Such a survey is underway involving a large statistical sampling of market beef, pork and chicken from numerous cities in the United States. Monitoring the levels of the parasite in actual market samples will involve several approaches, including bioassays, serum and tissue juice antibody assays and a molecular

assay developed with the support of National Pork Board funds. Samples are now being tested for T. gondii and the results for each test compared. These results will assure consumers of an accurate risk of exposure to toxoplasmosis through the commercial meat supply.

The immune system is complex and requires new tools to track the cells that respond to infectious organisms. Members of this laboratory participated in a major international workshop to determine the reactivity of ~200 monoclonal antibodies (mAb) against swine immune cells. The mAb were exchanged, tested for specific reactivity and the results statistically analyzed so that clusters of differentiation (CD) numbers could be assigned. The results are now internationally accepted as the standard CD designation for pig immune cells, enabling scientists worldwide to exchange data and determine which cells are essential in preventing infections or in stimulating protective vaccine responses. In 2003-2005 a screen of 386 anti-human CD mAb have been tested for cross-reactivity on swine cells as part of the Human Leukocyte Differentiation Antigens (HLDA8) workshop Animal Homologues Section. This will further increase availability of crucial anti-CD mAb for immune research in pigs.

Molecular markers of distinct Trichinella genotypes/species have been identified and developed into a sensitive diagnostic test. This test will reduce the risk of undetected freeze resistant Trichinella strains that could undermine the frozen meat technology that is the major commercial and consumer-based control strategy for the inactivation of Trichinella in edible tissue. In addition, the epidemiology of the remaining 9 other detectable genotypes can be monitored to prevent their emergence in the U.S.

Studies of toxoplasmosis have shown that strong protective immunity can be induced in pigs by irradiated oocysts and attenuated strains, but that immunity is not complete and may result in low level infection. Novel cytokine-based procedures that activate porcine immunity by increasing IFN-gamma levels have the potential to enhance resistance to toxoplasmosis. Details on the under developed nature of the intestinal immune system of weaned-pigs provides a model to test other prospective immune enhancing agents on development of resistance to infection in neonatal pigs.

Swine disease control is most important during the first month of a pig's life due to an immature immune system. ARS scientists have established methods to show how the respiratory and intestinal immune systems develop in the neonatal piglet and have shown that the immune system is under-developed in the intestine and, thus, less able to respond effectively against gastrointestinal infections. Future research should determine whether treatment of these young piglets with immune cytokine proteins will stimulate maturation of their immune system and thus enable them to be more resistant to infectious diseases as they move into the nursery, thus preventing piglet losses and saving producers' drug and veterinary costs.

ARS scientists at Beltsville, MD, with PIC/Sygen collaborators, have phenotyped hundreds of young piglets from PIC stock for early indicators of immune functions. This project was established to aid producers looking for alternatives to using antibiotics for early weaned pigs and researched methods to identify pigs which are genetically more disease resistant. The statistical analyses have indicated that some immune correlates of healthier and more productive

pigs do exist; further studies will determine whether healthier pigs can be identified using simpler genetic markers. With such markers, pigs which are more disease resistant could be made available so that consumers would be provided with healthier pork products that contained less drug residues.

New approaches are being explored to determine whether pigs that are genetically resistant to disease can be identified as an alternate to using antibiotics to treat early weaned pigs. ARS scientists at Beltsville, MD, in collaboration with PIC/Sygen have evaluated piglets' blood cell markers associated with immune function and showed that there were significant increases in specific T cell subsets during the first few weeks of a piglet's life. These results may help explain why neonates are more susceptible to disease and may also indicate how producers can plan management changes to avoid immune and disease stressors. Additionally this data indicated that there were piglets which were genetically more resilient, based on their growth characteristics and increased numbers of specific immune subsets at 7 weeks of age. Thus, genetic tests could be developed to identify breeders with this trait.

Pigs are exposed to infectious agents when they are moved to new facilities and mix with pigs from other areas. The use of antibiotics to control infection is limited and alternate therapeutic approaches are desperately needed. In collaboration with the Biotechnology Research and Development Corporation (BRDC) and their collaborating pharmaceutical and breeding company partners, ARS scientists cloned and purified a new pig therapeutic, recombinant porcine cytokine interleukin-12 (rPoIL-12). However, rPoIL-12 has limited activity in swine because of low IL-12 receptor expression [unlike other animal species] and therefore, may not, on its own, provide adequate stimulation of the pig immune system. This is essential information for pharmaceutical and vaccine companies as they search for effective immune stimulants for swine.

Modified live virus (MLV) vaccines are only partially effective in preventing new PRRSV infections. With BRDC funding ARS scientists and Univ. Illinois researchers performed detailed examination of immune gene expression following MLV vaccination and demonstrated that some innate immune responses were not stimulated by PRRS MLV. To improve these responses, they then tested innate adjuvants (interferon-alpha, IFNA, and interferon inducer poly(inosinic acid)-poly(cytidylic acid) [poly(IC)]) as adjuvants for MLV resulting in improved interferon-gamma production but limited increases in protection against PRRS. Thus, these new adjuvants will be helpful, but not sufficient, in improving PRRSV vaccines.

IMPACT:
A comprehensive panel of "designer" immune reagents has been developed to assess swine immune responses, for use in analyses of disease and vaccine studies and neonatal pig development. These include sensitive gene expression tests for >300 swine immune genes, mAb markers for swine cell subsets and immune cytokines, and biotherapeutics. Products from this CRIS's research will enhance an expanding base of scientific knowledge on mucosal and neonatal immunology of swine, techniques and reagents for veterinary application, practical outcomes for control of zoonotic pathogens and characterization of products with commercial applications.

Studies comparing pig immune gene expression to Toxoplasma gondii and Ascaris suum infections have shown that clearly distinct T helper I (Th1) and Th2 responses can be induced. Based on these gene expression studies swine researchers worldwide will be able to assess protective immunity to different infectious organisms and vaccinations. These gene expression assays will help determine whether novel cytokine-based procedures that activate porcine immunity have the potential to enhance resistance to specific infectious agents or development of resistance to infection in neonatal pigs.

This CRIS has developed new animal models to assess the impact of mixed infections on disease responses and on pig health. Testing responses to secondary bacteria disease in association with worm infection in livestock have indicated that the Th1/Th2 balance can be altered in local mucosal tissues. This has led to cooperative studies with human clinicians for inflammatory bowel syndrome and Crohn's disease.

This project has tested biotherapeutic alternatives to using antibiotics for early weaned pigs and researched methods to identify pigs which are genetically more disease resistant. The biotherapeutic porcine IL-12 protein was proven not to be effective. The adjuvants, IFNA and poly(IC) were partially effective as booster of anti-PRRS vaccine immunity. Studies proved that the respiratory and intestinal immune systems develop in the neonatal piglet is under-developed and, thus, less able to respond effectively against respiratory and gastrointestinal infections. Some immune correlates of healthier and more productive pigs do exist; studies are underway to determine whether healthier pigs can be identified using simpler genetic markers. With such adjuvants and markers, pigs which are healthier could be made available so that consumers would be provided with safer pork products that contained less drug residues.

Novel molecular parasite detection and diagnostic tests have been produced for swine parasites. Molecular markers of distinct Trichinella genotypes/species have been identified and developed into a sensitive diagnostic test that can be used for epidemiologic studies of the detectable Trichinella genotypes to prevent their emergence in the U.S. A real-time, PCR-based, sensitive diagnostic test to identify and quantitate Toxoplasma gondii DNA in tissues and other biological samples, including meat products, has been developed and a patent application.

TECHNOLOGY TRANSFER:
The results of these studies have been presented to a number of local groups at field days and presentations through the ARS National Visitor's Center and to scientific groups including the American Association of Veterinary Parasitologists, the World Association for the Advancement of Veterinary Parasitology, the Conference of Research Workers in Animal Disease, national and international animal genome meetings, and national and international immunology meetings. Scientists involved in this CRIS serve on the National Pork Board Toxoplasmosis Advisory group and assist in review of the Board's programs and grant submissions.

ARS scientists are also involved in multistate projects, NC-1004 (serving in 2004 as NC-1004 Chair) and NRSP8 for animal genome studies, and NC-229 for PRRS research. Most recently this has been evidenced by successful participation in the multistate USDA CSREES Animal and

Plant Biosecurity grant #2003-05164: "Integrated Control and Elimination of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in the U.S."

Reagents generated to study immune responses in swine have been made available to the research community worldwide. This has included molecular probes and real-time PCR primers and probes for swine cytokines and immune markers that have been shared internationally. Numerous monoclonal antibodies (mAb) reactive with swine immune cell subsets (CD antigens) and cytokines are now available through commercial sources. The results of international CD workshops are now internationally accepted, enabling scientists worldwide to exchange data and determine which cells are essential in preventing infections or in stimulating protective vaccine responses. Now, in 2003-2005, the workshop involves testing for cross-reactivity on swine cells as part of the Human Leukocyte Differentiation Antigens (HLDA8) workshop Animal Homologues Section. This will further increase availability of crucial anti-CD mAb for immune research in pigs.

The cloning and expression of a functional interleukin-12 (IL-12) cytokine was conducted under a BRDC funded grant. Sponsors from the BRDC legally reviewed the patent potential of porcine IL-12. No patent application was submitted. Availability of the cloned, expressed IL-12, is decided by BRDC.

Patents:
The patent application for the real-time, PCR-based, diagnostic test to identify Toxoplasma DNA in tissues and other biological samples, including meat products, was submitted to the US patent office. Further tests to determine usefulness on commercial pork products are underway through support of a new National Pork Board grant under the new CRIS project. Transfer of this technology to scientists at the Centers for Disease Control and other institutions has been ongoing .

PUBLICATIONS (Listed by CRIS objectives):

Objective #1: Develop reagents and assays to enable definition of cytokine-regulated immune mechanisms that protect pigs.

Dawson HD, Royaee AR, Nishi S, Kuhar D, Schnitzlein WM, Zuckermann F, Urban JF, Lunney JK. 2004. Identification of Key Immune Mediators Regulating T helper 1 Responses in Swine. Vet. Immunol. Immunopathol. In Press.

Royaee AR, Zuckermann F, Husmann R, Calzada-Nova G, Schnitzlein WM, Lunney JK. 2004. Cytokine response of porcine lymphoid cells in response to host vaccination with PRRS virus. Vet. Immunol. Immunopathol. In Press.

Zarlenga DS, Dawson H, Kringel H, Solano-Aguilar G, Urban JF Jr. 2004. Molecular Cloning of the Swine IL-4 receptor α and IL-13 receptor α1-chains: effects of experimental *Toxoplasma gondii, Ascaris suum* and *Trichuris suis* infections on tissue mRNA levels. Vet. Immunol. Immunopathol. In press.

Dawson HD, Beshah E, Nishi S, Solano-Aguilar G, Morimoto M, Zhao A, Madden KB, Ledbetter TK, Dubey JP, Shea-Donohue T, Lunney JK, Urban, JF Jr. 2004. Localized multi-gene expression patterns support an evolving Th1/Th2-like paradigm in response to infections with *Toxoplasma gondii* and *Ascaris suum* in pigs. Infection and Immunity. In Revision.

Martens GW, Lunney JK, Baker JE, Smith DM. 2003. Rapid Assignment of Swine Leukocyte Antigen (SLA) Haplotypes in Pedigreed Herds using a Polymerase Chain Reaction Based Assay. 2003. Immunogenetics. 55: 395-401.

Lunney JK, Fossum C, Alm GV, Steinbach F, Wattrang E. 2002. Veterinary immunology: opportunities and challenges. Trends in Immunology. 23: 4-6.

Solano-Aguilar G, Zarlenga D, Beshah E, Gasbarre L, Junker D, Cochran M, Dawson H, Urban J, Lunney J. 2002. Limited effect of Recombinant porcine Interleukin 12 on porcine lymphocytes due to a low level of IL-12 Beta 2 receptor. Vet. Immunol. Immunopathology. 89: 133-148.

Thacker E, Summerfield A, McCullough K, Ezquerra A, Dominguez J, Alonso F, Lunney J, Sinkora J, Haverson K. 2001. Summary of workshop findings for porcine myelomonocytic markers. Vet. Immunol. Immunopathology. 80:, 93-109.

Davis WC, Haverson K, Saalmüller A, Yang H, Lunney JK, Hamilton MJ, Pescovitz MD. 2001. Analysis of monoclonal antibodies reacting with molecules expressed on T cells. Vet. Immunol. Immunopathology. 80: 53-62.

Haverson K, Saalmüller A, Alvarez B, Alonso F, Bailey M, Bianchi ATJ, Boersma WJA, Chen Z, Davis WC, Dominguez J, Engelhardt H, Ezquerra A, Grosmaire LS, Hamilton MJ, Hollemweguer E, Huang C, Khanna KV, Kuebart G, Lackovic G, Ledbetter JA, Lee R, Llanes D, Lunney JK, McCullough KC, Molitor T, Nielsen J, Niewold TA, Pescovitz MD, Perez de la Lastra J, Rehakova Z, Salmon H, Schnitzlein WM, Seebach J, Simon A, Sinkora J, Sinkora M, Stocks CR, Summerfield A, Sver L, Thacker E, Valpotic I, Yang H, Zuckermann FA, Zwart R. 2001. Overview of the Third International Workshop on Swine Leukocyte Differentiation Antigens. Vet. Immunol. Immunopathol. 80: 5-23.

Johnson P, Keen NT, Lunney JK, Sadowsky MJ. 2001. Conference Editorial: Agricultural Microbes Genome 2. Comparative and Functional Genomics. v. 2. p. 10-13.

Objective #2: Determine if parasite-induced polarization of swine immunity effects pig health and susceptibility to zoonotic agents.

(Pig)

Kringel H, Dubey JP, Beshah E, Hecker R, Urban JF Jr. 2004. Effect of CpG oligodeoxynucleotide (ODN)-induced immune activation on porcine immunity to infection with *Toxoplasma gondii*. Vet. Parasitol. (in press).

Mansfield LS, Gauthier DT, Abner SR, Jones KM, Wilder SR Urban JF Jr. 2003. Enhancement of disease and pathology by synergy of *Trichuris suis* and *Campylobacter jejuni* in the colon of immunologically naive swine. Am J Trop Med Hyg. 68:70-80.

Abner SR, Hill DE, Turner JR, Black ED, Bartlett P, Urban JF Jr, Mansfield LS. 2002. Response of intestinal epithelial cells to *Trichuris suis* excretory-secretory products and the influence on *Campylobacter jejuni* invasion under in vitro conditions. J. Parasitol. 88: 738-745.

Solano-Aguilar GI, Beshah E, Vengroski K, Zarlenga D, Jauregui L, Cosio M, Douglass LW, Dubey JP, Lunney, JK. 2001. Cytokine and lymphocyte profile in miniature swine after oral infection with Toxoplasma gondii oocysts. Intnl J Parasitology. 31: 187-195.

Solano-Aguilar GI, Beshah E, Vengroski K, Zarlenga D, Jauregui L, Cosio M, Douglass LW, Dubey JP, Lunney JK. 2001. Cytokine and lymphocyte profile after oral infection with Toxoplasma gondii oocysts. Scandinavian J. Immunology. 54: 93.

(Mouse)
Ekkens M, Liu Z, Liu Q, Whitmire J, Xiao S, Foster A, Pesce J, VanNoy J, Sharpe A, Urban JF Jr., Gause WC. 2003. The role of OX40L interactions in the development of the Th2 response to the gastrointestinal nematode parasite Heligmosomoides polygyrus. J. Immunol 170:384-93.

Zhao A, McDermott J, Urban JF, Gause W, Madden KB, Au Yeung K, Morris SB, Finkelman FD, Shea-Donohue T. 2003. Dependence of IL-4, IL-13 or Nematode-Induced Alterations in Murine Small Intestinal Smooth Muscle Contractility on Stat6 and Enteric Nerves. J Immunol 171:948-954.

Strait RT, Morris SC, Smiley K, Urban JF Jr.,, Finkelman RD. 2003. IL-4 exacerbates anaphylaxis. J. Immunology. 170: 3835-3842.

Gause WC, Urban JF Jr., Stadecker MJ. 2003. The immune response to parasitic helminths: insights from murine models. Trends in Immunology. 24: 269-277.

Schopf LR, Hoffmann KF, Cheever AW, Urban JF Jr., Wynn TA. 2002. IL-10 is critical for resistance and survival during gastrointestinal helminth infection. J. Immunol. 168: 2383-2392.

Ekkens M, Liu Z, Liu Q, Foster A, Whitmire J, Pesce J, Urban JF Jr, Gause WC. 2002. Memory Th2 effector cells can develop in the absence of B7-1/B7-2, CD28 interactions, and effector Th cells after priming with an intestinal nematode parasite. J. Immunol. 168: 6344- 6351.

Madden KB, Whitman L, Sullivan C, Gause WC, Donaldson DD, Urban JF Jr., Katona IM, Finkelman FD, Shea-Donohue T. 2002. Role of Stat6 and mast cells in IL-4 and IL-13-induced alterations in murine intestinal epithelial cell function. J. Immunol. 169: 4417-4422.

Liu Z, Liu, Q., Pesce, J, Whitmire J, Ekkens M, Foster, A, VanNoy, J, Sharpe A, Urban JF Jr, Gause WC. 2002. *Nippostrongylus brasiliensis* can induce B7-independent Ag-specific

development of IL-4-producing T cells from naïve CD4 T cells in vivo. J. Immunol. 169: 6959-6968, 2002.

Finkelman FD, Urban JF Jr.. 2001. The other side of the coin: The protective role of the type 2 (Th2) cytokines. J. Allergy and Clinical Immunology. v.107. p.772-780.

Shea-Donohue T, Sullivan C, Finkelman FD, Madden KB, Morris SC, Goldhill J, Piñeiro-Carrero V, Urban JF Jr. 2001. The Role of Interleukin-4 in *Heligmosomoides polygyrus* induced alterations in murine intestinal epithelial cell function. J. Immunol. 167: 2234-2239.

Urban JF Jr., Noben-Trauth N, Schopf L, Madden K, Finkelman FD. 2001. Cutting edge: IL-4 receptor expression by non-bone marrow-derived cells is required to expel gastrointestinal nematode parasites. J. Immunol. 167: 6078-6081.

(Human)
Elliott DE, Li J, Blum A, Metwali A, Qadir K, Urban JF Jr, Weinstock JV. 2003. Exposure to Schistosome Eggs Protects Mice from TNBS-induced Colitis. Am J Physiol Gastrointest Liver Physiol. 284: G385-91.

Summers RW, Elliott DE, Qadir K, Urban JF Jr., Thompson R, Weinstock JV. 2003. *Trichuris suis* seems to be safe and possibly effective in the treatment of inflammatory bowel disease. Am. J. Gastroenterol. 98: 2034-2041.

Weinstock JV, Summers RW, Elliott DE, Qadir K, Urban JF Jr, Thompson R. 2002: The possible link between de-worming and the emergence of immunological disease. J Lab Clin Med. 139: 334-38.

Objective #3: Assess development of mucosal immune responses in pigs and the effect on neonatal pig health and transmission of foodborne pathogens using state-of-the-art immunological methods and cDNA microarray technology.

Zarlenga DS, La Rosa G, Pozio E, Rosenthal B. 2004. Identification and classification within the genus, *Trichinella*, with special emphasis on non-encapsulated species. Vet. Parasitol. (In press).

Morimoto M, Morimoto M, Whitmire J**,** Xiao S, Star RA, Urban JF Jr., Gause WC. 2004. Peripheral CD4 T cells rapidly accumulate at the host: parasite interface during an inflammatory Th2 memory response. J. Immunol. 172(4):2424-30.

Bannerman D, Paape MJ, Lunney JK. 2004. Immunity: Innate. In Encyclopedia of Animal Science; Pond WG, Bell AW Eds.; Marcel Dekker, Inc., New York. In Press.

Lunney JK, Paape MJ, Bannerman D. 2004.Immunity: Acquired. In Encyclopedia of Animal Science; Pond WG, Bell AW Eds.; Marcel Dekker, Inc., New York. In Press.

Gerrits RJ, Lunney JK, Johnson LA, Pursel VG, Rohrer GA, Dobrinsky JR. 2004. A vision for artificial insemination and genomics to improve the global swine population. Theriogenology. In Press.

La Rosa G, Marucci G, Zarlenga DS, Casulli A, Zarnke RL, Pozio E. 2003. A study of the ITS-2 locus in laboratory and natural isolates of *Trichinella nativa* and *Trichinella* T6 suggests an incipient genetic divergence between the two genotypes. Int. J. Parasitol. 33; 209-216.

Morimoto M, Zarlenga DS, Beard H, Alkharouf N, Matthews BF, Urban JF Jr. 2003. *Ascaris suum*: cDNA microarray analysis of 4th stage larvae (L4) during self-cure from the intestine. Exp. Parasitol. 104: 113-121.

Lunney, J.K. 2003. In Search of Disease-Resistant Pigs. National Hog Farmer, Apr 15, 2003, pp.30-34.

Lunney JK 2003. Are there immune gene alleles that determine whether a pig will be healthy? Genetics of Pig Health Symposium, M Boggess, Ed.; National Pork Board Press, DesMoines, IA. p.63-72.

Solano-Aguilar GI, Galina L, Douglass LW, Lunney JK. 2002. Developmental immunity in neonatal pigs monitored by the function of peripheral blood mononuclear cells. Proc. 17th Cong. Intnl. Pig Vet. Soc., Iowa. Vol. 1, p.259.

Dawson H, Nishi S, Beshah E, Solano-Aguilar G, Zarlenga D, Urban JF, Lunney JK. 2002. Functional genomic assessment of porcine disease resistance: real time assays of porcine immune gene expression. Proceedings 7th World Congress on Genetics Applied to Livestock Production. #13-41.

Pozio E, Zarlenga DS. La Rosa G. 2002. The detection of encapsulated and non-encapsulated species of *Trichinella* suggests the existence of two evolute lines in the genus. Parasite 8; 27-29.

Zarlenga DS, Boyd P, Lichtenfels JR, Hill D, Gamble HR. 2002. Identification and characterization of a cDNA sequence encoding a glutamic acid-rich protein specifically transcribed in *Trichinella spiralis* newborn larvae and recognized by infected swine serum. Int. J. Parasitol. 32; 1361-1370.

Solano-Aguilar GI, Vengroski K, Beshah E, Douglass LW, Lunney JK. 2001. Characterization of lymphocyte subsets from mucosal tissues in neonatal swine. Dev. Comp. Immunol. 25: 245-263.

Jauregui LH, Higgins J, Zarlenga D, Dubey JP, Lunney JK. 2001. Development of a Real-Time PCR Assay for Detection of *Toxoplasma gondii* in Pig and Mouse Tissues. J. Clin. Microbiol. 39: 2065-2071.

Zarlenga DS, La Rosa G. 2001. Traditional and Molecular methods for parasite differentiation within the genus *Trichinella.* Vet. Parasitol. 93; 279-292.

La Rosa G, Marucci G, Zarlenga DS, Pozio E. 2001. *Trichinella pseudospiralis* populations from the Palearctic region and their relationship with populations of Nearctic and Australian regions. Int. J. Parasitol. 31; 297-305.

Zarlenga DS, Higgins JA. 2001. PCR as a diagnostic and quantitative technique in veterinary parasitology. Vet. Parasitol. 101; 215-230.

Zarlenga DS, Chute MB, Martin A, Kapel CMO. 2001. A single multiplex PCR for differentiating all species of *Trichinella* with special application to the newly described *T. papuae.* Parasite 8; 24-26.

Animal Parasitic Diseases Laboratory (APDL)

Project Title: Biosystematics and Biodiversity of Pathogens and Parasites
CRIS Number: 1265-32000-068-00D
Scientists: Hoberg, E.P., Lichtenfels, J.R.
Location: Animal and Natural Resources Institute
Animal Parasitic Diseases Laboratory
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PROJECT OBJECTIVES:

Helminth parasites are economically significant pathogens in ruminants, other food animals and equines, contributing to over $2 billion in losses annually in the United States. Efforts to understand epidemiology, distribution and disease and the most efficacious basis for control have often been hampered by difficulties in identification of pathogens and parasites. A paucity of knowledge about the species and populations involved and often limited information on geographic and host-distribution hinders our ability to predict the outcome of host-parasite interactions. Long-term challenges are evident that require resolution even as we develop more refined morphological and molecular capabilities for parasite identification. Identity and phylogenetic relationship are baseline information for defining host and geographic distribution and patterns of parasite diversity. Recognition of cryptic, emergent, exotic and invasive pathogens, determination of the importance of reservoir hosts such as wildlife, and elucidation of the influence of interfaces between agricultural and wild ecosystems on the structure of helminth faunas follow from biodiversity research. Additionally, among ruminants, the mosaic nature of the helminth fauna, particularly hidden species diversity, impacts our abilities to recognize emerging pathogens and invasive species or those increasingly influenced by anthropogenic and climatological global change. Consequently, continued strategic biodiversity survey and inventory in conjunction with phylogenetic systematic studies of parasitic groups are necessary to provide for accurate identification, diagnostics and definition of the parasitic fauna. Ecological perturbation linked to global change and its influence on the distribution of pathogens and emergence of disease can only be understood with a context provided by systematics. These issues form the framework of the current program for a series of integrated studies in systematics and parasite biodiversity among the crown clades of the strongylate nematodes primarily in ruminants and equines. Concurrently we will expand, and curate the specimens-collections of the US National Parasite Collection (USNPC). An infrastructure for Biodiversity Bioinformatics will link interactive information systems and diagnostic identification keys, with phylogenetic and epidemiological information for dissemination on the internet. We also provide a resource for reference specimens and information to support parasitology and animal health nationwide and globally.

OVERALL PROJECT ACCOMPLISHMENTS:

(1) *Phylogenetic Studies Indicate That The Occurrence of Taenia Tapeworms* in humans predates the development of agriculture, animal husbandry and the domestication of cattle or swine. Taeniid tapeworms in African carnivores twice independently colonized hominids and the genus *Homo*, prior to the origin of modern humans coincidental with dietary and behavioral shifts from herbivory to scavenging and carnivory. Parasitological data provide a unique means of elucidating historical ecology, foraging behavior, and food habits of hominids during their evolution.

(2) *Comprehensive Taxonomic and Systematic Studies of Haemonchinae*- revealed 2 new species in *Haemonchus* and a new species of *Ashworthius*. The phylogeny for *Haemonchus* is the foundation for a comprehensive reference system for diagnostic criteria and development of interactive identification keys; historically, coevolutionary and biogeographic analyses demonstrate the pervasive role of host translocation, and parasite introduction/ emergence in the global distributions of *H. contortus*, *H. placei* and *H. similis* the dominant pathogenic species in domestic stock.

(3.) *Phylogeny for Nematodirinae Nematodes*- Nematodirinae are trichostrongyloid nematodes that globally include serious pathogens of ruminants, particularly caprines; completed the first phylogenetic analyses to reveal relationships among the 5 genera of nematodirines, and resolved historical biogeographic and host associations in the diversification of this parasite group.

(4.) *Mosaic Structure for Nematode Faunas in Ruminants from North America*- Phylogenetic and historical studies of the trichostrongyloids including Ostertagiinae, Nematodirinae, and Haemonchinae indicate dual origins for major components of the Nearctic fauna. The Arctic-boreal fauna and specifically the nematodrines, and many ostertagiines are endemic to North America, whereas the Haemonchines and Cooperiines have a history of introduction following Euopean contact in the 1500's. Empirical and theoretical studies designed to examine the role of dispersal and translocation on faunal structure indicate a complex history**.**

(5.) *Development and Application of Epizootiological Probes*- Protostrongylid muscleworms in the genus *Parelaphostrongylus* are emergent parasites among wild ruminant hosts in the continental United States. As requested by APHIS, and in collaboration with the College of Veterinary Medicine, Oregon State University, and the Western College of Veterinary Medicine, University of Saskatchewan, scientists of the US National Parasite Collection used comparative morphology, and multi-locus sequencing to identify larval parasites in feces of infected hosts and determine the geographic and host range for *P. odocoilei*. These studies indicated that a single species, *P. odocoilei* has a geographic distribution extending from California to south-central Alaska and the Mackenzie Mountains of the Northwest Territories and occurs in both Caprine and Cervid hosts; the parasite was identified for the first time in Oregon.

(6.) *Resources for Biodiversity Bioinformatics*- represents an effective and synergistic foundation to develop, utilize and present diverse knowledge about the distribution, host associations, and history for parasites represented by specimens-based collections such as the US National Parasite Collection. The Curator of the US National Parasite Collection in collaboration with colleagues at the Southwestern Museum of Vertebrate Biology, University of New Mexico,

the Western College of Veterinary Medicine, University of Saskatchewan, Department of Ecology and Evolutionary Biology, Cornell University, and the Vantaa Forest Research Centre, Rovaniemi, Finland developed protocols for comparative biodiversity studies focusing on parasite-host assemblages in high latitude ecosystems of the Holarctic. Studies serve as models for expansion of specimens-based collections as information systems integrating strategic field collections and databasing for development of comparative baselines for biodiversity in a contemporary and historical context.

(7.) *Molecular Diagnostics for Equine Strongyles*-in collaboration with scientists in Scotland, Australia, and Ukraine, methods for improved diagnostics, based on morphology and DNA probes, were developed for nematode pathogens in horses.

(8.) *Diagnostic Keys for Definitive Identification of Equine Strongyles*- in collaboration with V. Kharchenko of the Ukraine Academy of Science, and with support of a research grant from the CRDF (Civilian Research and Development Foundation), comparative morphological keys were prepared based on redescriptions of the 70 species of equine strongyles. Included were complete line figures and photomicrographs depiciting all significant diagnostic characters for identification. This work will remain the benchmark for all diagnostic work on the small strongyles, and is the foundation for any development of molecular-based probes for identification.

IMPACT:

(1.) The typical parasite fauna in humans is far older than previously considered and indicates a very prolonged association between humans and their characteristic food-borne pathogens. These insights provided a new focus in comparative epidemiological studies of *T. saginata*, *T. asiatica* and *T. solium* in humans and food animals for veterinarians and parasitolotigists in North America and globally.

(2.) Improved knowledge of the species composition of this economically important genus provides information critical to understanding patterns of host association and geographic distribution and contributes to the development of methods and agents for controlling these nematodes. This is the foundation for a synoptic on-line and interactive key for identification.

(3.) Information from these studies contributes to species-level analyses of *Nematodirus* and *Nematodirella*, including species known as serious pathogens in domestic sheep and cattle, and to improved diagnostics for these Holarctic nematodes. These studies are baselines in developing predictive hypotheses about the response of nematodes to changing climate and ecological perturbation. Essential and base-line information is disseminated to extension services, state departments of agriculture ands wildlife, and to the veterinary community.

(4.) Disparate origins and adaptations to climate reveal different capacities and responses are expected across the spectrum of nematode pathogens in ruminants within the context of global climate change. Expansion and population increases for temperate faunas including the highly pathogenic haemonchines is predicted. These insights are critical to any strategic planning

process for prediction of impacts linked to global climate change and the responses of complex host-parasite systems.

(5.) The investigation was the first to demonstrate the utility of "epizootiological probes" to elucidate the geographic and host associations for a pathogenic parasite; a baseline is provided to begin to understand the potential for emergence of such elaphostrongyline nematodes as a consequence of global change and the potential for host switching to domestic stock. These methods have broad applicability in both veterinary medicine and conservation biology, particularly where hosts cannot be collected for complete necropsy.

(6.) Specimens collections become portals for access to critical information linking interactive keys, phylogenetic, biological and epidemiological data through species homepages for helminth parasites in Holarctic mammals, including those that circulate among domestic and wild ruminants. Essentially for the veterinary and wildlife conservation communities this will represent "one-stop shopping" for access to definitive information to identify parasites, understand their host associations and geographic rtanges, and to predict the outcomes of interactions among wild and domestic hosts at the interface of natural and managed ecosystems.

(7.**)** Improved diagnostics provides essential working tools for the identification of species responsible for an emerging disease (larval cyathostomiasis), the development and testing of new antiparasitic compounds required by widespread nematode resistance to current drugs, and the testing of biological agents needed to control the more than 50 species of nematode pathogens in horses. The control of nematodes will greatly improve the health, well being and productivity of horses worldwide used for farming, recreation and in many countries for food.

(8.) Keys will be made available on line for access on the web. Such access will broadly disseminate the results of this research to the veterinary and equine communities and serve to standardize the basis for identifications of these pathogens.

TECHNOLOGY TRANSFER NP 103 (FY 2000-2004):

(1.) **US National Parasite Collection- database, informatics & specimens resources.** Internet website at: www.lpsi.barc.usda.gov/bnpcu. Serves as a primary resource for national and global parasitology. The USNPC is the largest specimens-based collection in the world. It serves a global constituency providing critical resources for systematics research, identification and diagnostics. It is a foundation for a network that provides proactive and predictive data for exotic, invasive and emergent pathogens and parasites.

BNPCU scientists provided a National Reference Collection of specimens of parasites in animals, through development of the US National Parasite Collection, and an associated database of specimen records to researchers worldwide via the Internet and the loan of specimens. **Impact:** Parasitologists in ARS, and others working in veterinary, medical, or wildlife parasitology, have access to the necessary specimens and database to conduct studies in identification, classification and distribution that impact all efforts to control parasites of agricultural or medical importance. The unique specimens, collected over a period of 150 years, provide a storehouse of genetic information that serves as a primary resource for biodiversity research. **Outcome:** The efficiency and prospects for success of all research worldwide on parasites of animals are enhanced. The identification and control of parasites that contaminate fish, shellfish, or red meat and/or increase the cost of its production is more accurate and efficient, resulting in food that is safer and less expensive. Prospects are enhanced for preventing the importation of exotic parasites that threaten American agriculture.

(2.) NSF Grant- Beringian Coevolution Project. (BS&I 0196095). 1999-2003. $240,000.

(3.) CRDF Grant, Cooperative Grants Program- Biological and Biomedical Engineering. Comparative morphology, phylogenetic analyses and classification of Strongyloid Nematode Parasites of Equidae. December 2001-2003. $80,000. (USNPC with Ukraine Academy of Sciences).

(4.) ARS-former Soviet Union Scientific Cooperation Program- Collaborative Grant. Population ecology, and systematics of the Family Protostrongylidae. February 2002-2005. (USNPC and Uzbekistan Academy of Sciences).

PUBLICATIONS- according to category in NP 103 (FY 2000-2004):

<u>Diagnostics</u> (including systematics, phylogeny, coevolution; empirical & theoretical studies; resources for systematics research; biodiversity)

BrooksDR, Dowling APG, van Veller MGP, Hoberg EP. Ending a decade of deception: A valiant failure, a not so valiant failure and a success story. Cladistics. 2004; 20: 32-46.

Brooks DR, Hoberg EP. Triage for the biosphere: The need and rationale for taxonomic inventories and phylogenetic studies of parasites. Comparative Parasitology. 2000; 67: 1-25. (order of authorship is arbitrary)

Brooks DR, Hoberg EP. Parasite systematics in the 21st Century: Opportunities and obstacles. Trends in Parasitology. 2001; 17: 273-275.

Dowling APG, van Veller MGP, Hoberg EP, Brooks, DR. A priori and a posteriori methods in comparative evolutionary studies of host-parasite associations. Cladistics. 2003; 19: 240-253.

Hoberg EP. Foundations for an integrative parasitology: collections archives and biodiversity informatics. Comparative Parasitology. 2002. v.69. p.124-131.

Hoberg EP. Colonization and diversification: Historical and coevolutionary trajectories among cestodes, pinnipeds and cetaceans. Proceedings International Congress of Parasitology X. Monduzzi Editore, Bologna. 2002. p. 65-69.

Hoberg EP. Coevolution in marine systems. *In* Marine Parasites, K. Rohde (ed.). CSIRO, Sydney, Australia. In Press.

Hoberg EP. Marine birds and their helminth parasites. *In* Marine Parasites, K. Rohde (ed.). CSIRO, Sydney, Australia. In Press.

Hoberg EP, Abrams A. Synlophe in *Ostertagia* cf. *kasakhstanica* (Nematoda: Ostertagiinae), the minor morphotype of *O. bisonis* in western North America. Journal of Parasitology. 2001; 87: 1181-1184.

Hoberg, E.P., Abrams, A., Carreno, R., Lichtenfels, J.R. *Ashworthius patriciapilittae* n. sp. (Trichostrongyloidea: Haemonchinae), an abomasal nematode in *Odocoileus virginianus* from Costa Rica, and a first record for the genus in the Western Hemisphere. Journal of Parasitology. 2002; 88: 1187-1199.

Hoberg EP, Adams A. Phylogeny, history and biodiversity: Understanding faunal structure and biogeography in the marine realm. Bulletin of the Scandinavian Society of Parasitology. 2000; 10: 19-37.

Hoberg, E.P., Klassen, G.J. Revealing the faunal tapestry: Coevolution and historical biogeography of hosts and parasites in marine systems. Parasitology. 2002; Supplement 124: S3-S22.

Hoberg EP, Lichtenfels JR, Gibbons LM. Phylogeny for species of the genus *Haemonchus* (Nematoda: Trichostrongyloidea): Considerations of their evolutionary history and global biogeography among Camelidae and Pecora (Artiodactyla). Journal of Parasitology; 2004; 90: In Press.

Hoberg EP, Mariaux J, Brooks DR. Phylogeny among orders of the Eucestoda (Cercomeromorphae): Integrating morphology, molecules and total evidence. In: Interrelationships of the Platyhelminthes. DTJ Littlewood and RA Bray (eds). Taylor and Francis. 2001; pp. 112-126.

Hoberg EP, Rickard L, Lichtenfels JR. Phylogeny for the Nematodirinae (Nematoda: Trichostrongyloidea): Coevolution and historical biogeography among Lagomorpha and Artiodactyla. Journal of Parasitology. 2004; 90: In Press.

Hodgkinson, J.E., Love, S., Lichtenfels, J.R., Palfreman, S., Ramsey, Y.H., and Matthews, J.B. Evaluation of the specificity of five oligoprobes for identification of cyathostomin species from horses. International Journal of Parasitology. 2001; 31:197-204.

Kharchenko, V.A., Lichtenfels, J.R. and Krecek, R.C. *Cyathostomum montgomeryi* and its place in the Cyathostominae (Nematoda: Strongylidae). Comparative Parasitology. 2001. 68:97-102.

Lichtenfels, J.R., Gibbons, L.M., Krecek, R.C. Recommended terminology and advances in the systematics of the Cyathostominea (Nematoda; Strongyloidea) of horses. Veterinary Parasitology. 2003; 107: 337-342.

Lichtenfels, J.R., McDonnell, A., Love, S. and Matthews, J.B. Nematodes of the Tribe Cyathostominea (Strongylidae) collected from horses in Scotland. Comparative Parasitology. 2001; 68: 265-269.

Lichtenfels, J.R. and Pilitt, P.A. Synlophe patterns of the Haemonchinae of ruminants (Nematoda: Trichostrongyloidea). Journal of Parasitology. 2000. 86:1093-1098.

Lichtenfels, J.R., Pilitt, P.A., Gibbons, L.M.and Boomker, J.D.F. *Haemonchus horaki* n. sp. (Nematoda: Trichostrongyloidea) from the rhebok, *Pelea capreolus*, in South Africa. Journal of Parasitology. 2001; 87: 1095-1103.

Lichtenfels, J.R., Pilitt, P.A., Gibbons, L.M., Hoberg, E.P. Redescriptions of *Haemonchus mitchelli* and *Haemonchus okapiae* (Nematoda: Trichostrongyloidea) and descriptions of a unique synlophe for the Haemonchinae. Journal of Parasitology. 2002; 88: 947-960.

McDonnell, A., Love, S., Tait, A., Lichtenfels, J.R. and Matthews, J.B. Phylogenetic analysis of partial mitochondrial cytochrome oxidase C subunit I and large ribosomal RNA sequences and nuclear internal transcribed spacer 1 sequences from species of Cyathostominae and Strongylinae (Nematoda, Order Strongylida) parasites of the horse. Parasitology. 2000; 121: 649-659.

Murrell, K.D., Lichtenfels, J.R., Zarlenga, D.S. and Pozio, E. The systematics of the genus *Trichinella* with a key to species. Veterinary Parasitology. 2000; 93: 293-307.

Nadler SA, Hoberg EP, Hudspeth DSS, Rickard LG. Relationships of *Nematodirus* species and *Nematodirus battus* isolates (Nematoda: Trichostrongyloidea) based on nuclear ribosomal DNA sequences. Journal of Parasitology. 2000; 86: 588-601.

Rickard L, Hoberg EP. Reassignment of the genus *Lamanema* (Nematoda: Trichostrongyloidea) from Nematodirinae to Molineinae. Journal of Parasitology. 2000; 86: 647-650.

Santin-Duran M, Alunda JM, de la Fuente C, Rosenthal B, Hoberg EP. Identical ITS-1 and ITS-2 sequences indicate that *Spiculopteragia asymmetrica* and *S. quadrispiculata* (Nematoda: Trichostrongylidae) constitute morphologically distinct variants of a single species. Journal of Parasitology. 2002; 88: 417-418.

Zamparo D, Brooks DR, Hoberg EP, McLennan, DA. Phylogenetic analysis of the Rhabdoceola (Platyhelminthes) with emphasis on the Neodermata and relatives. Zoologica Scripta. 2001; 30: 59-77.

Zoonotic Pathogens

Andersen, E.M. and Lichtenfels, J.R. Anisakiasis. In: W.M. Meyers, ed., Pathology of Infectious Diseases. Volume I Helminthiases. Armed Forces Inst. Pathology, Washington, DC. 2000. 529pp.

Hoberg, E.P. *Taenia* tapeworms: biology, evolution and socioeconomic significance. Microbes and Infection. 2002; 4: 859-866.

Hoberg EP, Alkire NL, de Queiroz A, Jones A. Out of Africa: Origins of the *Taenia* tapeworms in humans. Proceedings Royal Society of London, Series B. 2001; 268: 781-787.

Hoberg EP, Jones A, Rausch RL, Eom KS, Gardner SL. A Phylogenetic hypothesis for species of the genus *Taenia* (Eucestoda: Cyclophyllidea). Journal of Parasitology. 2000; 86: 89-98.

Wünschmann A, Garlie V, Averbeck G, Kurtz H, Hoberg EP. Cerebral cysticercosis by *Taenia crassiceps* in a domestic cat. Journal of Veterinary Diagnostic Investigation. 2003; 15: 484-488.

Epidemiology of Disease & Global Climate Change

Hoberg EP, Kocan A, Rickard LG. Gastrointestinal strongyles in wild ruminants. In: Parasitic Diseases of Wild Mammals. W Samuel, M Pybus and A Kocan (eds). Iowa State University Press. 2001; pp. 193-227.

Hoberg, E.P., Kutz, S.J., Cook, J., Galbreath, K. Arctic biodiversity: From discovery to faunal baselines - revealing the history of a dynamic ecosystem.Journal of Parasitology. 2003; 89: S84-S95.

Hoberg EP, Kutz S, Nagy J, Jenkins E, Elkin B, Branigan M, Cooley D. *Protostrongylus stilesi* (Protostrongylidae): Ecological isolation and putative host switching between muskoxen and Dall's sheep in a contact zone. Comparative Parasitology. 2002; 69: 1-9.

Kutz SJ, Hoberg EP, Polley L. Emergence of third stage larvae of *Umingmakstrongylus pallikuukensis* from three gastropod intermediate host species. Journal of Parasitology. 2000; 86: 743-749.

Kutz SJ, Hoberg EP, Polley L. *Umingmakstrongylus pallikuukensis* (Nematoda: Protostrongylidae) in gastropods: Larval morphology, morphometrics, and development rates. Journal of Parasitology. 2001; 87: 527-535.

Kutz S, Hoberg EP, Polley L. A new lungworm in muskoxen: An exploration in Arctic parasitology. Trends in Parasitology. 2001; 17: 276-280.

Kutz SJ, Hoberg EP, Polley L. Development of *Umingmakstrongylus pallikuukensis* in a gastropod intermediate host under Arctic field conditions. Canadian Journal of Zoology. 2002; 80: 1977-1985.

Santin-Duran M, Alunda JM, de la Fuente C, Hoberg EP. Onchocercosis in red deer (Cervus elaphus) from Spain. Journal of Parasitology. 2001; 87: 1213-1215.

Santin-Duran M, Alunda JM, San Miguel JM, Hoberg EP, de la Fuente C. Elaeophorosis in red deer (*Cervus elaphus*) from central Spain. Journal of Wildlife Diseases. 2000; 36: 779-782.

Santin-Duran M, Alunda JM, Hoberg EP, de la Fuente C. Abomasal parasites in wild sympatric cervids, red deer *Cervus elaphus* and fallow deer *Dama dama*, at three locations from Central and Western Spain. Journal of Parasitology 2004; 90: In Press.

Emerging, Exotic, Invasive Pathogens

Carreno R, Durden L, Brooks DR, Abrams A, Hoberg EP. *Parelaphostrongylus tenuis* (Nematoda: Protostrongylidae) and other parasites of white-tailed deer (*Odocoileus virginianus*) in Costa Rica. Comparative Parasitology. 2001; 68: 177-184.

Kutz SJ, Hoberg EP, Nagy J, Polley L. Emerging parasitic infections in Arctic ungulates. American Zoologist. 2004; 44: In Press.

Kutz SJ, Veitch A, Hoberg EP, Elkin B, Jenkins EJ, Polley L. New host and geographic records for the protostrongylid nematodes *Protostrongylus stilesi* and *Parelaphostrongylus odocoilei* in Dall's sheep (*Ovis dalli dalli*). Journal of Wildlife Diseases. 2001; 37: 761-774.

Immunology

Zarlenga, D.S., Boyd, P., Lichtenfels, J.R., Hill, D., Gamble, H.R. Identification and characterization of a cDNA sequence encoding a glutamic acid rich protein specifically transcribed in *Trichinella spiralis* newborn larvae and recognized by infected swine serum. International Journal for Parasitology. 2002; 32.1361-1370.

ABSTRACTS- NP 103 (FY 2000-2004):

(invited abstracts)

Hoberg EP, Schauff M. USDA Collections at the interface of agriculture and the environment. Association of Systematics Collections, National Conference. Symposium, Information, Technology, and Partnerships: Natural History Collections in the New Millennium. Baltimore, Maryland. 13-15 May 2000.

Hoberg EP. Phylogeny, history and biodiversity: Understanding faunal structure and biogeography in the marine realm. *In*: Ecological Parasitology on the Turn of the Millennium. Zoological Institute, Russian Academy of Sciences & Scandinavian Society for Parasitology, St. Petersburg, Russia. July 2000.

Hoberg EP. Biodiversity and Biogeography: Establishing the context and keystones for Arctic parasitology. General Introduction. International Workshop for Arctic Parasitology, Prince Albert National Park, Saskatchewan, Canada. October 2000.

Kutz S, Hoberg EP, Polley L. Global warming and host-parasite systems in the Arctic: Should we be concerned? Issues Presentation. International Workshop for Arctic Parasitology, Prince Albert National Park, Saskatchewan, Canada. October 2000.

Jenkins E, Hoberg EP, Kutz S, Veitch A, Polley L. Challenges in investigating protostrongylids in northern hosts: *Parelaphostrongylus odocoilei* in Dall's sheep (*Ovis dalli dalli*) of northern North America. Issues Presentation. International Workshop for Arctic Parasitology, Prince Albert National Park, Saskatchewan, Canada. October 2000.

Hoberg EP. Foundations for an integrative parasitology: collections archives and biodiversity informatics. *In*: Symposium- Parasitology in Science and Society. Helminthological Society of Washington, 676th Meeting, and Smithsonian Institution, Washington D.C. 27 October 2001.

Cook J, Galbreath K, Runck A, Fedorov V, Hoberg EP. Beringian Coevolution Project: An interface between mammalogy and parasitology. *In*: Biogeography and Evolution in Northern Host-Parasite Systems: A Symposium Honoring Robert L. Rausch and Virginia R. Rausch. International Congress of Parasitology X. Vancouver, Canada. 4-9 August 2002.

Hoberg EP. Colonization and diversification: Historical and coevolutionary trajectories among cestodes, cetaceans and pinnipeds. *In*: Symposium for Parasites of Marine Mammals. International Congress of Parasitology X. Vancouver, Canada. 4-9 August 2002.

Hoberg EP, Kutz SJ, Cook J. Arctic biodiversity: From discovery to faunal baselines- revealing the history of a dynamic ecosystem. SubPlenary Lecture, *In*: Parasitology and Biodiversity in an Age of Discovery. International Congress of Parasitology X. Vancouver, Canada. 4-9 August 2002.

Hoberg EP. Robert and Virginia Rausch: Fifty years on the frontiers of northern parasitology. *In*: Biogeography and Evolution in Northern Host-Parasite Systems: A Symposium Honoring Robert L. Rausch and Virginia R. Rausch. International Congress of Parasitology X. Vancouver, Canada. 4-9 August 2002.

Hoberg EP, Alkire N, de Queiroz A, Jones A. Phylogeny of *Taenia* tapeworms: Insights about the origins of species in humans. *In*: Symposium on *Taenia asiatica* and cysticercosis. International Congress of Parasitology X. Vancouver, Canada. 4-9 August 2002.

Hoberg EP. Hominids, carnivores and *Taenia* tapeworms: Revealing historical associations for parasites and pathogens in humans.*In*: Biologie des cestodes: recherches actuelles. IIIe Cycle Romand en Sciences Biologiques. Muséum d'Histoire naturelle de Geneve. 1-3 October 2002.

Hoberg EP. Old worms in new hosts? Radiation of the Tetrabothriidea in marine birds and mammals. *In*: Biologie des cestodes: recherches actuelles. IIIe Cycle Romand en Sciences Biologiques. Muséum d'Histoire naturelle de Geneve. 1-3 October 2002.

Kutz SJ, Hoberg EP. Stress in the Arctic- Emerging parasitic infections of arctic ungulates. *In*: Biology of the Canadian Arctic: A crucible for change in the 21st Century. Society of Integrative and Comparative Biology. Toronto, Canada. 4-9 January 2003.

Jenkins E, Veitch AM, Appleyard GD, Hoberg EP, Kutz SJ, Polley L. Geographic distribution and seasonal patterns of larval shedding of the muscle-dwelling nematode *Parelaphostrongylus odocoilei* in thinhorn sheep from northern North America. 15th Annual Arctic Forum, Arctic Research Consortium of the United States. 28-29 April 2003. (*ARCUS Award for Research Excellence- Life Sciences*).

Hoberg EP. Collections, archives and informatics: A new synergy for tracking history and biodiversity from species to populations. International Workshop for Arctic Parasitology II. Arctic Centre, University of Lapland, Rovaniemi, Finland. 1-7 September 2003.

Kutz SJ, Hoberg EP, Nagy J, Elkin B, Veitch A, Jenkins E, Polley L. Emerging parasitic infections and diseases of Arctic ungulates. International Workshop for Arctic Parasitology II. Arctic Centre, University of Lapland, Rovaniemi, Finland. 1-7 September 2003.

Hoberg EP. Tapeworm evolution: Insights on phylogeny and history in the global biosphere. Plenary Lecture. International Science Conference, Dedicated to Academician Konstantin Ivanovich Skrjabin on the Occasion of His 125th Anniversary. Laboratory of Helminthology, Institute of Parasitology, Russian Academy of Sciences. Moscow, 14-16 April 2004. (Abstract published; visa denied).

(contributed abstracts)

Hoberg EP. Invasive and emerging pathogens: Biodiversity at the interface of agricultural and natural ecosystems. Invasive Species Symposium. Agricultural Research Service, Beltsville, Maryland. 15 February 2000.

Kutz S, Hoberg EP, Polley L. Parasite biodiversity, development and transmission: An integrative model for monitoring climate change at northern latitudes. 12th Northern Wild Sheep and Goat Conference, Whitehorse, Yukon Territory, Canada. May 2000.

Kutz S, Veitch A, Hoberg EP, Elkin B, Jenkins E, Polley L. New host and geographic records for *Protostrongylus stilesi* and *Parelaphostrongylus odocoilei* in Dall's sheep from the Mackenzie Mountains, NWT. 12th Northern Wild Sheep and Goat Conference, Whitehorse, Yukon Territory, Canada. May 2000.

Kutz SJ, Hoberg EP, Polley L. A model for investigating the development, transmission, and response to climate change of protostrongylid parasites on the Arctic tundra. Wildlife Disease Association, Annual Meeting. Jackson, Wyoming. 4-8 June 2000.

Kutz SJ, Veitch A, Jenkins E, Elkin B, Chirino-Trejo M, Hoberg E, Polley L. *Parelaphostrongylus odocoilei* and *Protostrongylus stilesi* in Dall's sheep: Predisposing factors for mortality? Wildlife Disease Association, Annual Meeting. Jackson, Wyoming. 4-8 June 2000.

Hoberg EP, Rosenthal B. Biosystematics and faunal assessment of parasites in Holarctic Bovidae and Cervidae. International Workshop for Arctic Parasitology, Prince Albert National Park, Saskatchewan, Canada. 1-4 October 2000.

Galbreath K, Hoberg EP, Telford S, Cherniavsky F, Kutz S, Duszynski D, Waltari E, Cook J. Current status of the Beringian Coevolution Project. International Workshop for Arctic Parasitology, Prince Albert National Park, Saskatchewan, Canada. October 2000.

Hoberg EP, Kutz SJ. Arctic biodiversity: baselines, archives and host-parasite systems as a window on faunal change. Taxonomy, Systematics, Phylogeny. American Society of Parasitologists, 76th Annual Meeting, Albuquerque, New Mexico. June 2001.

Lichtenfels JR, Pilitt PA, Gibbons LM, Hoberg EP. Synlophe pattern differences among species of *Haemonchus* (Nematoda: Trichostrongyloidea).Taxonomy, Systematics, Phylogeny. American Society of Parasitologists, 76th Annual Meeting, Albuquerque, New Mexico. June 2001.

Kutz SJ, Veitch A, Nagy J, Elkin B, Hoberg EP, Jenkins E, Polley L. Contact zones between expanding muskoxen populations and Dall's sheep- An emerging disease issue.13th Biennial Northern Wild Sheep and Goat Council Symposium. Rapid City, South Dakota. April 2002.

Jenkins E, Veitch A, Hoberg EP, Kutz SJ, Elkin B, Polley L. Distribution, life cycle and significance of *Parelaphostrongylus odocoilei* in a new host- Thinhorn sheep. 13th Biennial Northern Wild Sheep and Goat Council Symposium. Rapid City, South Dakota. April 2002.

Kutz SJ, Veitch A, Simmons N, Hoberg EP, Elkin B, Jenkins E, Polley L. Parasites in Dall's sheep: What can we learn from historical and contemporary collections (Or: Putting together the pieces).13th Biennial Northern Wild Sheep and Goat Council Symposium. Rapid City, South Dakota. April 2002.

Jenkins E, Veitch A, Hoberg EP, Kutz S, Elkin B, Polley L. Life cycle, distribution and significance of *Parelaphostrongylus odocoilei* in thinhorn sheep (*Ovis dalli*).World Conference on Mountain Ungulates III. Zaragosa, Spain. June 2002.

Kutz SJ, Nagy J, Elkin B, Hoberg EP, Wagner B, Polley L. Epidemiology of abomasal parasites of muskoxen on Banks Island, Northwest Territories. World Conference on Mountain Ungulates III. Zaragosa, Spain. June 2002.

Kutz SJ, Veitch A, Simmons N, Hoberg EP, Elkin B, Jenkins E, Polley L. Parasites in Dall's sheep: What can we learn from historical and contemporary collections? World Conference on Mountain Ungulates III. Zaragosa, Spain. June 2002.

Kutz SJ, Nagy J, Elkin B, Hoberg EP, Gasbarre L, Wagner B, Polley L. Epidemiology of abomasal parasites of muskoxen on Banks Island, Northwest Territories, Canada. Wildlife Disease Association 51st Annual Meeting. Arcata, California. July 2002.

Wagner B, Bollinger T, Hoberg EP. Case Report: Subcutaneous *Taenia crassiceps* cysticercosis in an Arctic fox (*Alopex lagopus*). Wildlife Disease Association 51st Annual Meeting. Arcata, California. July 2002.

Kutz SJ, Hoberg EP, Cook J, Simmons N, Nagy J, Veitch A, Elkin B, Polley L. Parasitism in wildlife populations: Baselines, biodiversity and monitoring in the Arctic. Wildlife Parasitology Session, International Congress of Parasitology X. Vancouver, Canada. 4-9 August 2002.

Kutz SJ, Jenkins E, Hoberg EP, Appleyard G, Veitch A, Nagy J, Nishi J, Elkin B, Mulders R, Rosenthal B, Polley L. Ungulate parasitology in the Canadian north. 11th Arctic Ungulate Conference, Saariselkä, Finland. 24-28 August 2003.

Kutz SJ, Nagy J, Elkin B, Hoberg EP, Gasbarre L, Veitch A, Polley L. The role of gastrointestinal parasitism in muskox population dynamics. 11th Arctic Ungulate Conference, Saariselkä, Finland. 24-28 August 2003.

Kutz SJ, Appleyard G, Jenkins E, Scheer A, Nagy J, Veitch A, Elkin B, Cooley D, Johnson D, Hoberg E, Polley L. Assessing, predicting, and monitoring the impact of climate change on infectious diseases in caribou: What do we know and what do we need to know. 10th North American Caribou Conference, Fairbanks, Alaska. May 2004.

Kutz SJ, Jenkins E, Veitch A, Nishi J, Elkin B, Polley L, Hoberg E. Climate warming and emerging infectious diseases in Arctic and Subarctic wildlife. 38th Annual Congress of the Canadian Meteorological and Oceanographic Society (CMOS), Edmonton, Alberta. 31 May- 3 June 2004.

Jenkins E, Veitch A, Appleyard G, Hoberg E, Ogunremi D, Kutz S, PolleyL. A newly recognized neurological disease associated with *Parelaphostrongylus odocoilei* in experimentally infected thinhorn sheep. Wildlife Disease Association, 53rd Annual Meeting (with American Association of Zoo Veterinarians and American Association of Wildlife Veterinarians) Captive and Free Ranging Wildlife: Health and Conservation. San Diego, California 27 August- 3 September 2004.

Animal Parasitic Diseases Laboratory (APDL)

Title: Biology and Epidemiology of *Neospora caninum* and Related Protozoa

CRIS No.: 1265-32000-071-00D
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PROJECT OBJECTIVES:

Neosporosis, caused by *Neospora caninum*, is a major cause of abortion in cattle, and it also causes paralysis in other animals. It is distributed worldwide. *Sarcocystis neurona* is the most important cause of neurologic illness in horses in the United States. The objective of research conducted on these parasites include preventing transmission of *Neospora* to dairy and beef cattle, and preventing transmission of *S. neurona* from opossums to horses. For each parasite, understanding their biology and epidemiology will lead to improved control.
A conservative estimate for losses associated with *Neospora*-induced abortion in dairy cattle in California alone is approximately 35 million dollars annually, not accounting for the cost of culling the cows, re-breeding the cows, and loss of milk production. *Sarcocystis neurona* is the most important cause of neurologic disease in horses. It causes over 100 million dollars in annual losses to the equine industry. Basic information is needed on the biology and epidemiology of these parasites so that control programs based on management or vaccine strategies may be designed. This project is related to Animal Health NP 103, Component 2-Pathogen Detection and Diagnostics, Component 4-Animal Immunology, Component 5-Mechanisms of Disease, Component 7-Epidemiology of Disease, and Component 8-Strategies to Control Infectious and Non-infectious Disease. *Neospora* and *Sarcocystis neurona* are newly recognized and emerging pathogens. This research supports national program priorities for parasites including improved pathogen detection, monitoring and surveillance of disease, mechanisms of disease and disease prevention and control.

OVERALL PROJECT ACCOMPLISHMENTS:

Neospora caninum, a major cause of abortion in cattle, was identified and named. Epidemiology of *N. caninum* in dogs and bovines was investigated. Molecular methods have been developed to differentiate *N. caninum* oocysts from oocysts of other coccidian parasites that utilize dogs as the definitive host. This will allow for specific detection of dogs infected with *N. caninum* and will advance the elucidation of the life cycle of the parasite within the dog definitive host. Molecular studies have been conducted to identify strain differences between tissue cysts and tachyzoites of *N. caninum* isolated from different geographic regions. These studies will determine if immunization protocols and detection assays developed from one geographic isolate will be useful for isolates from other regions. *Neospora caninum* infection was found in dogs even in remote areas of the world such as the Amazon, Brazil. Studies have been conducted to determine the life cycle and identify stages of the parasite in naturally congenitally infected puppies. *Neospora caninum* was isolated for the first time from an aborted bovine fetus in Portugal and

neosporosis was documented as the most important cause of bovine abortion in Portugal. The pathogenesis of *N. caninum* infection and abortion is largely unknown. Initial studies were conducted in pregnant cows inoculated with *N. caninum* at 110 day of gestation. Studies have demonstrated placental invasion of the parasite in infected, pregnant cows. The placental invasion by the parasite and down regulation of cytokines appears to be an important pathogenic mechanism.

Diagnostic tests and animal models were developed to screen drugs and vaccines. A reproducible model of congenital neosporosis was developed in mice such that female mice infected with the parasite during gestation produce offspring that are infected with *N. caninum*. This mouse model reproduces one major clinical feature of neosporosis in cattle and now provides a means of testing vaccines against the parasite that may be used to prevent the disease in cattle. Another important accomplishment was the complete protection against neosporosis afforded by immunization of mice with *N. caninum* tachyzoite antigen. Complete protection was obtained against congenital transfer of *Neospora caninum* in mice by immunization with plasmid DNA coding for a tachyzoite dense granule protein. This result points to studies that may be conducted in cattle and possibly reveal individual antigens that may elicit a protective response. Significant protection was elicited against *N. caninum*-induced abortion in sheep by immunization of ewes with native *N. caninum* tachyzoite antigen emulsified in a proprietary adjuvant.

Sarcocystis neurona, the cause of neurologic diseases in horses and other animals and fatal encephalomyelitis in horses was named, cultured, diagnostic tests were developed, and its source of infection (the opossum) was identified. The complete life cycle of *S. neurona* was documented in a natural intermediate host, the raccoon. Fatal neurologic *S. neurona* infection was documented in a cat following a routine surgical operation to remove claws suggesting that stress can reactivate this disease. The equine model of *S. neurona* infection in horses was refined which results in production of clinical sarcocystosis in the horse. The development of this model will allow studies to determine the biology and progression of infection leading to disease in the horse. As few as 100 sporocysts caused clinical sarcocystosis in horses. Methods to kill *S. neurona* sporocysts in the environment were studied and moist heat at 60^{o}C killed sporocysts.

Besnoitiosis is a disease related to sarcocystosis. It causes morbidity and mortality in livestock. A new species of *Besnoitia oryctofelisi* was discovered and its life cycle completed in the laboratory. The ultrastructure of *Besnoitia* stages was studied to aid diagnosis.

IMPACT:

TECHNOLOGY TRANSFER:
The results of these studies have been presented to a number of local, national, and international scientific and industry groups including the American Association of Veterinary Parasitologists, the World Association of Veterinary Parasitologists, ARPAS, and other groups.

Reagents and information supplied to drug companies formed the basis for development and successful marketing of diagnostic kits and vaccines for neosporosis (for example, Iddex ELISA kit for *Neospora*).

PUBLICATIONS:

Dubey, J.P., Venturini, L., Venturini, M.C., and Speer, C.A. Isolation of *Sarcocystis speeri* Dubey and Lindsay, 1999 from the South American opossum (*Didelphis albiventris*) from Argentina. J. Parasitol. **86:**160-163, 2000.

Speer, C.A., Dubey, J.P. and Mattson, D.E. Comparative development and merozoite production of two isolates of *Sarcocystis neurona* and *Sarcocystis falcatula* in cultured cells. J. Parasitol. **86:**25-32, 2000.

Dubey, J.P., Lindsay and D.S. Gerbils (*Meriones unguiculatus*) are highly susceptible to oral infection with *Neospora caninum* oocysts. Parasitol. Res. **86:**165-168, 2000.

Lindsay, D.S. and Dubey, J.P. Determination of the activity of diclazuril against *Sarcocystis neurona* and *Sarcocystis falcatula* in cell cultures. J. Parasitol. **86:**164-166, 2000.

Lindsay, D.S., Thomas, N.J. and Dubey, J.P. Biological characterization of *Sarcocystis neurona* from a Southern sea otter (*Enhydra lutris nereis*). Int. J. Parasitol. **30:**617-624, 2000.

Duncan, R.B., Fox, J.H., Lindsay, D.S., Dubey, J.P. and Zuccaro, M.E. Acute sarcocystosis in white-tailed deer (*Odocoileus virginianus*). J. Wildlife Dis. **36:**357-361, 2000.

Lindsay, D.S. and Dubey, J.P. Canine neosporosis. J. Vet. Parasitol. **14:**1-11, 2000. (Review).

Dubey, J.P., Lindsay, D.S., Venturini, L. and Venturini, C. Characterization of *Sarcocystis falcatula* isolates from the Argentinian opossum, *Didelphis albiventris*. J. Eukaryot. Microbiol. **47:**260-263, 2000.

Dubey, J.P. *Neospora caninum*: un agent abortif majeeur des bovins. Bulletin des GTV **7:**85, 2000. (Editorial).

Dubey, J.P., Speer, C.A., Bowman, D.D., Horton, K.M., Venturini, C. and Venturini, L. Experimental transmission of *Sarcocystis speeri* Dubey and Lindsay, 1999 from the South American opossum (*Didelphis albiventris*) to the North American opossum (*Didelphis virginiana*). J. Parasitol. **86:**624-627, 2000.

Dyer, R.M., Jenkins, M.C., Kwok, O.C.H., Douglas, L.W. and Dubey, J.P. Serologic survey of *Neospora caninum* infection in a closed dairy cattle herd in Maryland: risk of serologic reactivity by production groups. Vet. Parasitol. **90:**171-181, 2000.

Dubey, J.P., Speer, C.A. and Lindsay, D.S. In vitro cultivation of schizonts of *Sarcocystis speeri* Dubey and Lindsay, 1999. J. Parasitol. **86:** 671-678, 2000.

Dubey, J.P. Prevalence of *Sarcocystis* species sporocysts in wild caught opossums (*Didelphis virginiana*). J. Parasitol. **86:**705-710, 2000.

Dubey, J.P., Clark, T.R. and Yantist, D. *Frenkelia microti* infection in a chinchilla (*Chinchilla laniger*) in the United States. J. Parasitol. **86:**1149-1150, 2000.

Dubey, J.P., Garner, M.M., Rosenthal, B.M. and DeGhetto, D. Clinical coccidiosis in raccoons (*Procyon lotor*). J. Parasitol. **86:** 1299-1303, 2000.

Lindsay, D. S., Dubey, J. P., and Kennedy, T. J. Determination of the activity of ponazuril against *Sarcocystis neurona* in cell cultures. Vet. Parasitol. **92:** 165-169, 2000.

Lindsay, D. S., Dykstra, C. C., Williams, A., Spencer, J. A., Lenz, S. D., Palma, K., Dubey, J. P., and Blagburn, B. L. Inoculation of *Sarcocystis neurona* merozoites into the central nervous system of horses. Vet. Parasitol. **92:** 157-163, 2000.

Dubey, J. P. and Hamir, A. N. Immunohistochemical confirmation of *Sarcocystis neurona* infections in raccoons, mink, cat, skunk and pony. J. Parasitol. **86:** 1150-1152, 2000.

Lindsay, D. S., McKown, R. D., and Dubey, J. P. *Sarcocystis campestris* from naturally infected 13-lined ground squirrels, *Spermphilus tridecemlineatus tridecemlineatus*, from Nebraska. J. Parasitol. **86:** 1159-1161, 2000.

Dubey, J. P., Lindsay, D. S., Rezende, P. C. B., and Costa, A. J. Characterization of an unidentified *Sarcocystis falcatula*-like parasite from the South American opossum, *Didelphis albiventris* from Brazil. J. Eukaryot. Microbiol. **47:** 538-544, 2000.

Frenkel, J. K. and Dubey, J. P. The taxonomic importance of obligate heteroxeny: distinction of *Hammondia hammondi* from *Toxoplasma gondii* - another opinion. Parisitol.Res. **86:** 783-786, 2000.

Schares, G., Rausner, M., Söndgen, P., Rehberg, P., Bärwald, A., Dubey, J. P., Edelhofer, R., and Conraths, F. J. Use of purified tachyzoite surface antigen p38 in an ELISA to diagnose bovine neosporosis. Int. J. Parasitol. **30:** 1123-1130, 2000.

Dubey, J. P., Saville, W. J. A., Lindsay, D. S., Stich, R. W., Stanek, J. F., Speer, C. A., Rosenthal, B. M., Njoku, C. J., Kwok, O. C. H., Shen, S. K., and Reed, S. M. Completion of the life cycle of *Sarcocystis neurona* . J. Parasitol. **86:** 1276-1280, 2000.

Dubey, J. P., Kerber, C. E., Lindsay, D. S., Kasai, N., and Pena, H. F. J. The South American opossum, *Didelphis marsupialis*, from Brazil as another definitive host for *Sarcocystis speeri* Dubey and Lindsay, 1999. Parasitology **121:** 589-594, 2000.

Cummings, C. A., Kocan, A. A., Barker, R. W., and Dubey, J. P. Muscular sarcocystosis in coyotes from Oklahoma. J. Wildlife Dis. **36:** 761-763, 2000.

Jenkins, M. C., Caver, J. A., Björkman, C., Anderson, T. C., Romand, S., Vinyard, B., Uggla, A., Thulliez, P., and Dubey, J. P. Serological investigation of an outbreak of *Neospora caninum*-associated abortion in a dairy herd in southeastern United States. Vet. Parasitol. **94:** 17-26, 2000.

Dubey, J. P., Quist, C. F., and Fritz, D. L. Systemic sarcocystosis in a wild turkey. J. Wildlife Dis. 36: 755-760. 2000.

Dubey, J. P., Fritz, D., Lindsay, D. S., Shen, S. K., Kwok, O. C. H., and Thompson, K. C. Diclazuril preventive therapy of gamma interferon knockout mice fed *Sarcocystis neurona* sporocysts. Vet. Parasitol. **94:** 257-263, 2001.

Dubey, J. P., Lindsay, D. S., Saville, W. J. A., Reed, S. M., Granstrom, D. E., and Speer, C. A. A review of *Sarcocystis neurona* and equine protozoal myeloencephatitis (EPM). Vet. Parasitol. **95:** 89-131, 2001. (Review).

Rosenthal, B. M., Lindsay, D. S., and Dubey, J. P. Relationships among *Sarcocystis* species transmitted by New World opossums (*Didelphis* spp.). Vet. Parasitol. **95:** 133-142, 2001.

Dubey, J. P., Mattson, D. E., Speer, C. A., Hamir, A. N., Lindsay, D. S., Rosenthal, B. M., Kwok, O. C. H., Baker, R. J., Mulrooney, D. M., Tornquist, S. J., and Gerros, T. C. Characterization of a recent isolate of *Sarcocystis neurona* (SN7) from a horse and loss of pathogenicity of isolates SN6 and SN7 by passages in cell culture. Vet. Parasitol. **95:** 155-166, 2001.

Lindsay, D. S. and Dubey, J. P. Direct agglutination test for the detection of antibodies to *Sarcocystis neurona* in experimentally infected animals. Vet. Parasitol. **95:** 179-186, 2001.

Saville, W. J. A., Stich, R. W., Reed, S. M., Njoku, C. J., Oglesbee, M. J., Wunschmann, A., Grover, D. L., Larew-Naugle, A. L., Stanck, J. F., Granstrom, D. E., and Dubey, J. P. Utilization of stress in the development of a model for equine protozoal myeloencephalitis. Vet. Parasitol. **95:** 211-222, 2001.

Speer, C. A. and Dubey, J. P. Ultrastructure of schizonts and merozoites of *Sarcocystis neurona.* Vet. Parasitol. **95:** 263-272, 2001.

Dubey, J. P., Black, S. S., Rickard, L. G., Rosenthal, B. M., Lindsay, D. S., Shen, S. K., Kwok, O. C. H., Hurst, G., and Rashmir-Raven, A. Prevalence of *Sarcocystis neurona* sporocysts in opossums (*Didelphis virginiana*) from rural Mississippi. Vet. Parasitol. **95:** 283-293, 2001.

Dubey, J. P., Lindsay, D. S., Kerber, C. E., Kasai, N., Pena, H. F. T., Kwok, O. C. H., Shen, S. K., and Rosenthal, B. M. First isolation of *Sarcocystis neurona* from the South American opossum, *Didelphis albiventris*, from Brazil. Vet. Parasitol. **95:** 283-293, 2001.

Hamir, A. N. and Dubey, J. P. Myocarditis and encephalitis associated with *Sarcocystis neurona* infection in raccoons (*Procyon lotor*). Vet. Parasitol. **95:** 335-340, 2001.

Dubey, J. P. Migration and development of *Sarcocystis neurona* in tissues of interferon gamma knockout mice fed sporocysts from a naturally infected opossums. Vet. Parasitol. **95:** 341-351, 2001.

Dubey, J.P., Liddell, S., Mattson, D., Speer, C.A., Howe, D.K., Jenkins, M.C. Characterization of the Oregon isolate of *Neospora hughesi* from a horse. J. Parasitol. **87:** 345-353, 2001.

Hill, D.E., Liddell, S., Jenkins, M.C., Dubey, J.P. Specific detection of *Neospora caninum* oocyst in fecal samples from experimentally-infected dogs using the polymerase chain reaction. J. Parasitol. **87:** 395-398, 2001.

Basso, W., Venturini, L., Venturini, M.C., Hill, D.E., Kwok, O.C.H., Shen, S.K., Dubey, J.P. First isolation of *Neospora caninum* from the feces of a naturally infected dog. J. Parasitol. **87:** 612-618, 2001.

Lindsay, D.S., Dubey, J.P. Determination of the activity of pyrantel tartrate against *Sarcocystis neurona* in gamma-interferon gene knockout mice. Vet. Parasitol. **97:** 141-144, 2001.

Lindsay, D.S., Thomas, N.J., Rosypal, A.C., Dubey, J.P. Dual *Sarcocystis neurona* and *Toxoplasma gondii* infection in a Northern sea otter from Washington state, USA. Vet. Parasitol. **97:** 319-327, 2001.

Basso, W., Venturini, L., Venturini, M.C., Moore, P., Rambeau, M., Unzaga, J.M., Campero, C., Bacigalupe, D., Dubey, J.P. Prevalence of *Neospora caninum* infection in dogs from beef-cattle farms, dairy farms, and from urban areas of Argentina. J. Parasitol. **87:** 906-907, 2001.

Dubey, J.P., Rosenthal, B.M., Speer ,C.A. *Sarcocystis lindsayi* n. sp. (Protozoa: Sarcocystidae) from the South American opossum, *Didelphis albiventris* from Brazil. J. Eukaryot. Microbiol. **48:** 595-603, 2001.

Fujii, T.U., Kasai, N., Nishi, S.M., Dubey, J.P., Gennari, S.M. Seroprevalence of *Neospora caninum* in female water buffaloes (*Bubalus bubalis*) from the southeastern region of Brazil. Vet. Parasitol. **99:** 331-334, 2001.

Dubey, J.P., Saville, W.J.A., Stanek, J.F., Lindsay, D.S., Rosenthal, B.M., Oglesbee, M.J., Rosypal, A.C., Njoku, C.J., Stich, R.W., Kwok, O.C.H., Shen, S.K., Hamir, A.N., Reed, S.M. *Sarcocystis neurona* infections in raccoons (*Procyon lotor*): evidence for natural infection with sarocysts, transmission of infection to opossums (*Didelphis virginiana*), and experimental induction of neurologic disease in raccoons. Vet. Parasitol. 100**:** 117-119, 2001.

Dubey, J.P., Lindsay, D.S., Kwok, O.C.H., Shen, S.K. The gamma interferon knockout mouse model for *Sarcocystis neurona*: comparison of infectivity of sporocysts and merozoites and routes of inoculation. J. Parasitol. **87:** 1171-1173, 2001.

Dubey, J.P., Lindsay, D.S., Fritz, D., Speer, C.A. Structure of *Sarcocystis neurona* sarcocysts. J. Parasitol. 87**:** 1323-1327, 2001.

Dubey, J.P., Rosypal, A.C., Rosenthal, B.M., Thomas, N.J., Lindsay, D.S., Stanek, J.F., Reed, S.M., Saville, W.J.A. *Sarcocystis neurona* infections in sea otter (*Enhydra lutris*): evidence for natural infections with sarcocysts and transmission of infection to opossums (*Didelphis virginiana*). J. Parasitol. **87:** 1387-1393, 2001.

Dubey, J.P., Lindsay, D.S., Rosenthal, B.M., Kerber, C.E., Kasai, N., Pena, H.F.J., Kwok, O.C.H., Shen, S.K., Gennari, S.M. Isolates of *Sarcocystis falcatula*-like organisms from South American opossums *Didelphis marsupialis* and *Didelphis albiventris* from Sao Paulo, Brazil. J. Parasitol. **87:** 1449-1453, 2001.

Dubey, J.P., Johnson, G.C., Bermudez, A., Suedmeyer, K.W., Fritz, D.L. Neural sarcocystosis in a straw-necked ibis (*Carphibis spinicollis*) associated with a *Sarcocystis neurona*-like organism and description of muscular sarcocysts of an unidentified *Sarcocystis* species. J. Parasitol. **87:** 1317-1322, 2001.

Fayer, R., Trout, J.M., Xiao, L., Morgan, U.M., Lal, A.A., Dubey, J.P. *Cryptosporidium canis* n. sp. from domestic dogs. J. Parasitol. **87:** 1415-1422, 2001.

Lindsay, D.S., Rosypal, A.C., Spencer, J.A., Cheadle, M.A., Zajac, A.M., Rupprecht, C., Dubey, J.P., Blagburn, B.L. Prevalence of agglutinating antibodies to *Sarcocystis neurona* in raccoons, *Procyon lotor*, from the United States. Vet. Parasitol. **100:** 131-134, 2001.

Rickard, L.G., Black, S.S., Rashmir-Raven, A., Hurst, G., Dubey, J.P. Risk factors associated with the presence of *Sarcocystis neurona* sporocysts in opossums (*Didelphis virginiana*). Vet. Parasitol. **102:** 179-184, 2001.

Dubey,J.P. Recent development in the biology of Sarcocystis in the biology of *Sarcocystis neurona* and equine protozoal myeloencephalitis (EPM). J.Vet. Parasitol. **15:** 91-102, 2001.

Dubey,J.P.. Parasitemia and early tissue localization of *Sarcocystis neurona* in interferon gama gene knockout mice fed sporocysts. J. Parasitol. **87**: 1476-1479, 2001

Dubey, J.P., Hill, D., Lindsay, D.S., Jenkins, M.C., Uggla, A., and Speer, C.A. *Neospora caninum* and *Hammondia heydorni* are separate species. Trends Parasitol. **18:** 66-69, 2002.

Venturini, L., Petrucce1li, M.., Piscopo, M., Unzaga, J.M., Venturini, M.C.,Bacigalupe, D., Basso, W., Dubey,J..P. Natural Besnoitia sp. infection in domestic rabbits from Argentina. Veterinary Parasitology. **107:** 273- 278, 2002.

Dubey, J.P., Eggers, J.S., Lipscomb, T.P. Intestinal coccidiosis in a spinner dolphin (Stenella longirostris) .Journal of Parasitology. **88:** 634- 637, 2002.

Kinne, J., Ali, M., Wernery,U., Dubey, J.P. Clinical large intestinal coccidiosis in camels (Camelus dromedarius) in the United Arab Emirates: description of lesions, endogenous stages, and redescription of Isospora orlovi, Tsygankov, 1950 oocysts. Journal of Parasitology. **88:** 548-552, 2002.

Ordeix, L., Lloret,A., Fondevila, D., Dubey, J.P., Ferrer, L., Fondati,A. Cutaneous neosporosis during treatment of pemphigus foliaceus in a dog. Journal of the American Animal Hospital Association. **38:** 415-419, 2002.

Canada, N., Meireles, C.S.,Rocha, A., Sousa, S., Thompson, G., Dubey, J.P.,Romand, S., Thulliez, P., Correia da Costa, J.M. First Portuguese isolate of Neospora caninum from an aborted fetus from a dairy herd with endemic neosporosis. Veterinary Parasitology. **110:** 11-15, 2002.

Buxton, D., McAllister, M., Dubey, J.P. The comparative pathogenesis of neosporosis. Trends in Parasitology. **18:** 546-552, 2002.

Dubey, J.P., Lindsay, D.S.,Hill, D., Romand, S., Thulliez, P., Kwok, O.C.H.,Silva, J.C.R., Oliveira-Camargo, M.C., Gennari, S.M. Prevalence of antibodies to Neospora caninum and Sarcocystis neurona in sera of domestic cats from Brazil. Journal of Parasitology. **88:** 1251-1252, 2002.

Dubey, J.P., Saville, W.J.,Sreekumar, C., Shen, S.K., Lindsay, D.S., Pena, H.F.,Vianna, M.C., Gennari, S.M., Reed, S.M. Effects of high temperature and disinfectants on the viability of Sarcocystis neurona sporocysts. Journalof Parasitology. **88:** 1252-1254, 2002.

Sofaly, C.D., Reed, S.M., Gordon, J.C., Dubey, J.P., Oglesbee, M.J., Njoku, C.J.,Grover, D.L., Saville, W.J.~. Experimental induction of equine protozoal myeloencephalitis (EPM) in the horse: effect of Sarcocystis neurona sporocyst inoculation dose on the developmentof clinical neurologic disease. Journal of Parasitology. **88:** 1164-1170, 2002.

Stanek, J.F., Dubey, J.P., Oglesbee, M.J., Reed, S.M., Lindsay, D.S., Capitini, L.A., Njoku, C.J., Vitt~tow, K.J., Saville, W.J.A. Life cycle of Sarcocystis neurona in its natural intermediate host, the racoon, Procyon lotor. Journal of Parasitology. **88:** 1151-1158, 2002.

O'Handley, R., Liddell, S., Parker, C., Jenkins, M.C., Dubey, J.P. Experimental infection of sheep with Neospora caninum oocysts. Journal of Parasitology. **88:** 1120-1123, 2002.

Gennari, S.M., Yai, L.E., D'Auria, S.N., Cardoso, S.M., Kwok, O., Jenkins, M.C., and Dubey, J.P. Occurrence of *Neospora caninum* in sera from dogs of the city of Sao Paolo, Brazil. Vet. Parasitol. **106:** 177-179, 2002.

Dubey, J.P., Sreekumar, C.,Lindsay, D.S., Hill, D., Rosenthal, B.M., Venturini,L., Venturini, M.C., Greiner, E.C. Besnoitia oryctofelisi n. sp. (Protozoa: Apicomplexa) from domestic rabbits. Parasitology. **126:** 521-539, 2002.

Resendes, A.R., Juan-Salles, C., Almeria, S., Maj6, N., Domingo, M., Dubey, J.P. Hepatic sarcocystosis in a striped dolphin (Stenella coeruleoalba) from the Spanish Mediterranean coast. Journal of Parasitology. **88:** 206- 209, 2002.

Dubey, J.P., Barr, B.C., Barta, J.R., Bjrekas, I., Bjorkman, C., Blagburn, B.L., Bowman, D.D., Buxton, D., Ellis, J.T., Gottstein, B., Hemphill, A., Hill, D.E., Howe, D.K., Jenkins, M.C., Kobayashi, B., Marsh, A.E., Mattsson, J.G., McAllister, M.M., Modry, D., Omata, Y., Sibley, L.D., Speer, C.A., Trees, A.J., Uggla, A., Upton, S.J., Williams, D.J., and Lindsay, D.S. Redescription of *Neospora caninum* and its differentiation from related coccidia. Int. J. Parasitol. **32:** 929-946, 2002.

Silva, D.S., Bahia-Oliveira, L.M.G., Shen, S.K., Kwok, O.C.H., Lehmann, T., Dubey,J.P. Prevalence of Toxoplasma gondii in chickens from an area in southern Brazil highly endemic to humans. Journal of Parasitology. **89:** 394-396, 2003.

Dubey JP, Shkap V, Pipano E, Fish L, Fritz DL. Related Articles, Links Ultrastructure of Besnoitia besnoiti tissue cysts and bradyzoites.J Eukaryot Microbiol. **50:** 240-244, 2003.

Lindsay DS, Collins MV, Mitchell SM, Cole RA, Flick GJ, Wetch CN, Lindquist A, Dubey JP. Sporulation and survival of Toxoplasma gondii oocysts in seawater.J Eukaryot Microbiol. **50:** 687-688.

Dubey JP, Cawthorn RJ, Speer CA, Wobeser GA. Related Articles, Links Redescription of the sarcocysts of Sarcocystis rileyi (Apicomplexa: Sarcocystidae).J Eukaryot Microbiol. **50:** 476-82, 2003.

Burns R, Williams ES, O'Toole D, Dubey JP. Toxoplasma gondii infections in captive black-footed ferrets (Mustela nigripes), 1992-1998: clinical signs, serology, pathology, and prevention.J Wildl Dis. **39:** 787-797, 2003.

Sreekumar C, Graham DH, Dahl E, Lehmann T, Raman M, Bhalerao DP, Vianna MC, Dubey JP. Genotyping of Toxoplasma gondii isolates from chickens from India. Vet Parasitol. **118:** 187-194, 2003.

Dubey JP, Navarro IT, Graham DH, Dahl E, Freire RL, Prudencio LB, Sreekumar C, Vianna MC, Lehmann T. Related Articles, Links Characterization of Toxoplasma gondii isolates from free range chickens from Parana, Brazil. Vet Parasitol. **117:** 229-234, 2003.

Dubey JP, Sreekumar C. Related Articles, Links Redescription of Hammondia hammondi and its differentiation from Toxoplasma gondii. Int J Parasitol. 33: 1437-1453, 2003.

O'Handley, R.M., Morgan, S.A., Parker, C., Jenkins, M.C.and Dubey, J.P. Vaccination of ewes for prevention of vertical transmission of *Neospora caninum*. Am. J. Vet. Res. 64: 449-452, 2003.

Liddell, S., Parker, C., Vinyard, B., Jenkins, M.C., and Dubey, J.P. Immunization of mice with plasmid DNA coding for NcGRA7 or NcsHSP33 confers partial protection against congenital neosporosis. J. Parasitol. **89:** 496-500, 2003.

Dubey JP, Salant H, Sreekumar C, Dahl E, Vianna MC, Shen SK, Kwok OC, Spira D, Hamburger J, Lehmann TV. High prevalence of Toxoplasma gondii in a commercial flock of chickens in Israel, and public health implications of free-range farming.Vet Parasitol. **121:** 317-322, 2004.

Dubey JP, Graham DH, De Young RW, Dahl E, Eberhard ML, Nace EK, Won K, Bishop H, Punkosdy G, Sreekumar C, Vianna MC, Shen SK, Kwok OC, Sumners JA, Demarais S, Humphreys JG, Lehmann T. Related Articles, Links Molecular and biologic characteristics of Toxoplasma gondii isolates from wildlife in the United States. J Parasitol. **90:** 67-71, 2004.

Jenkins M, Parker C, Tuo W, Vinyard B, Dubey JP. Related Articles, Links Inclusion of CpG adjuvant with plasmid DNA coding for NcGRA7 improves protection against congenital neosporosis.Infect Immun. **72:** 1817-1819, 2004.

Sreekumar, C., Hill, D.E., Miska, K.B., Rosenthal, B.M., Vianna, M.C.B., Venturini, L., Basso, W., Gennari, S.M., Lindsay, D.S., and Dubey, J.P. *Hammondia heydorni*: Evidence of Genetic Diversity Among Isolates from Dog. Experimental Parasitology, *in press*.

Canada, N., Meireles, C.S., Mezo, M., Warleta, M., Correia da Costa, J.M., Sreekumar, C., Hill, D.E., Miska, K., and Dubey, J.P. First Isolation of *Neospora caninum* from an aborted bovine fetus in Spain. Journal of Parasitology, *in press*.

Jenkins, M.C., Tuo, W., Dubey, J.P. Vaccination of sheep with *Neospora caninum* protein protects against fetal loss associated with experimental neosporosis. Am. J. Vet. Res. , *in press*.

Proceedings and Abstracts :

Frenkel, J.K. and Dubey, J.P. Taxonomic importance of obligate heteroxeny and the distinction of *Hammondia hammondi*. Proc. Am. Soc. Parasit. , San Juan, Puerto Rico, 2000, Abstract No. 55.

Hill, D.E. and Dubey, J.P. Detection of *Neospora caninum* oocyst DNA in dog feces. Proc. Am. Soc. Parasit. , San Juan, Puerto Rico, 2000.

Fujii, T.U., Kasai, N., Gennari, S.M., Baruselli, P.S. and Dubey, J.P. Seroprevalence of anti-*Neospora caninum* antibodies in water buffaloes from Vale do Ribeira de Iguape, Sao Paulo State, Southeastern Brazil. Proc. Am. Soc. Parasit., San Juan, Puerto Rico, 2000. Abstract No. 189.

Heckeroth, A.R., Dubey, J.P., Conrad, P.A., Sverlow, K., Uggla, A., Hemphill, A., Müller,N. and Tenter,A.M. Comparison and devaluation of diagnostic PCRs for *Neospora caninum* and other Toxoplasmatinae. 12th Japanese-German Symposium on Protozoan Diseases, Bonn, 2000.

Hollis, K.M., Anchor, C., Chelsvig, J., Etter, D.R., Dubey, J.P., Warner, R.E. and Hungerford, L.L. Radio-telemetry and geographical information systems to assess urban deer zoonoses. 62nd Midwest Fish and Wildlife Conference, Minneapolis, MN, December 2000.

Lindsay, D.S., Thomas, N.J., Rosenthal, B.M. and Dubey, J.P. Biological characterization of *Sarcocystis neurona* isolated from a Southern sea otter (*Enhydra lutris nereis*). Proc. Ann. Meet. Am. Assoc. Vet. Parasit., Salt Lake City, Utah, 2000. Abstract No. 34.

O'Handley, R.M., Jenkins, M.C., Bjorkman, C., Uggla, A. and Dubey, J.P. Identification of immunodominant antigens recognized by cattle with acute and chronic *Neospora caninum* infections associated with abortion. Proc. Ann. Meet. Am. Assoc. Vet. Parasit. , Salt Lake City, Utah, 2000. Abstract No. 33.

Heckeroth, A. R., Dubey, J. P., Conrad, P. A., Sverlow, K., Uggla, A., Hemphill, A., Müller, N., and Tenter, A. M. Comparison and devaluation of diagnostic PCRs for *Neospora caninum* and other Toxoplasmatinae. 12th Japanese-German Symposium on Protozoan Diseases, Bonn. 2000.

Heckeroth, A. R., Dubey, J. P., Kwok, O. C. H., Conrad, P. A., Sverlow, K., Uggla, A., Hemphill, A., Müller, N., and Tenter, A. M., 2000. Vergleich und Evaluierung diagnostischer PCRs im Hinblick auf Spezifität für *Neospora caninum* und Verwandte Arten Zystenbildender Kokzidien. Diagnostik und Epidemiologie der *Neospora caninum*- Infektion des Rindes Workshop, Kurzfassungen der Vorträge, 23.-24.11.2000.

Dubey, J.P. Gamma interferon knockout mouse model for *Sarcocystis neurona*. American Association of Veterinary Parasitologists 46th Annual Meeting. Boston, MA, 2001. Abstract No. 28.

Rosypal, A.C., Lindsay, D.S., Duncan Jr, R.B., Ahmed, S.A., Zajac, A.M., Dubey, J.P. Sporocyst induced *Sarcocystis neurona* infections in mice lacking the gene for inducible nitric oxide synthase. American Association of Veterinary Parasitologists 46th Annual Meeting. Boston, MA, 2001. Abstract No. 30.

O' Handley, R.M., Morgan, S.A., Parker, C., Jenkins, M.C., McCrary, J., Kwok, O., Dubey, J.P. *Neospora* vaccination in an ovine model. American Association of Veterinary Parasitologists 46th Annual Meeting. Boston, MA, 2001. Abstract No. 32.

Rosenthal, B.M. and Dubey, J.P. Genetic diversity of *Sarcocystis* species that employ opossums as their definitive host. American Society of Parasitologists 76th Annual Meeting. Albuquerque, NM, 2001. Abstract No. 116.

Dubey, J.P. A review of *Neospora caninum* in cattle. Proc. III Congresso Ibérico de Reproduçno Animal July 5th-8th. Fundaçno Cupertino Miranda-Porto, 2001. Abstract No. 25.

Souza, S.L.P., Guimarnes JR, J.S., Dubey, J.P., Gennari, S.M. PrevalLncia de anticorpos anti-*Neospora canium* em cnes de propriedades produtoras de leite b da regino norte do estado do paraná. Jornal Brasileiro de Patologia, **37,** 2001. Abstract No. 98.

Rosypal, A.C., Lindsay, D.S., Duncan Jr, R.B., Ahmed, S.A., Zajac, AM., Dubey, J.P. Role of nitric oxide in resistance to sporocyst induced *Sarcocystis neurona* infections in mice. 53rd Annual Meeting of the Animal Disease Research Workers in Southern States, Gainesville, FL, 2001. Abstract No. 26.

Dubey, J.P. A review of neosporosis in cattle. The 10th International Congress of Parasitology, Vancouver, Canada, 2002. Abstract No. PL1-04.

Jenkins, M.C., O'Handley, R., O'Brian, C., Dubey, J.P. Cloning of a *Neospora caninium* apical complex antigen that may differentiate point source from reactivated bovine neosporosis. The 10th International Congress of Parasitology, Vancouver, Canada, 2002. Abstract No. PA-121

Almeria, S., De Marez, T., Dawson, H., Araujo R., Padilla, T., Dubey, J.P., Gasbarre, L.C. Immune responses in dam and fetus after experimental infection of pregnant cows with *Neospora caninum*. The 10th International Congress of Parasitology, Vancouver, Canada, 2002. Abstract No. S14-3-B.

Sofaly, C.D., Reed, S.M., Gordon, J.C., Dubey, J.P., Saville, W.J.A. Effects of *Sarcocystis neurona* sporocyst inoculation dose on the induction of equine protozoal myeloencephalitis (EPM). Proceedings of the American Association of Veterinary Parasitologists 47th Annual Meeting, Nashville, TN, 2002. Abstract No. 21.

Yai, L.E.O., Joppert, A.M., CaZón Franco, W.A., Souza L.P., Dubey, J.P., Gennari, S.M. OcorLncia de anticorpos anti-*Neospora caninum* em gambás (*Didephis* ssp.) do município de Sno Paulo, Brasil. 12th Congresso Brasileiro de Parasitologia Veterinária, Rio de Janeiro, Brazil, 2002. Poster No. P-066.

De Souza, S.L.P., Rossi, F.W., Dos Santos, L.C., Yai, L.E.O., CaZón Franco, W.A., Dubey, J.P., Gennari, S.M. OcorLncia de anticorpos anti-*Neospora caninum* em Cervídeos (*Mazama*

govasoubira) do Brasil.12th Congresso Brasileiro de Parasitologia Veterinária, Rio de Janeiro, Brazil, 2002. Poster No. P-133.

Da Silva Guimarnes JR, J., Souza, S.L.P., Bergamaschi, D.P., Dubey, J.P., Jenkins, M.C., Gennari, S.M. *Neospora caninum* em bovinos de exploraçno leiteira tipo B no norte do paraná, Brasil: Comparaçno entre trLs mLtodos diagnósticos.Brasil.12th Congresso Brasileiro de Parasitologia Veterinária, Rio de Janeiro, Brazil, 2002. Poster No. P-134.

Da Silva Guimarnes JR, J., Souza, S.L.P., Bergamaschi, D.P., Dubey, J.P., Gennari, S.M. *Neospora caninum* em bovinos de exploraçno leiteira tipo B no norte do paraná, Brasil: Estudo da prevalLncia e de fatores de risco. 12th Congresso Brasileiro de Parasitologia Veterinária, Rio de Janeiro, Brazil, 2002. Poster No. P-135.

CaZón Franco, W.A., Silva, J.C.R., Pinter, A., Labruna, M.B., Camargo, L.M.A. de Camargo, E.P., Bergamaschi, D.P., Dubey, J.P., Gennari, S.M. Validade do teste de aglutinaçno direta (NAT/MAT) no dignóstico de anticorpos anti-*Neospora caninum* e *Toxoplasma gondii* em Cnes. 12th Congresso Brasileiro de Parasitologia Veterinária, Rio de Janeiro, Brazil, 2002. Poster No. P-147.

CaZón Franco, W.A., Silva, J.C.R., Pinter, A., Souza, S.L.P., Labruna, M.B., Camargo, L.M.A., Camargo, E.P., Bergamaschi, D.P., Dubey, J.P., Gennari, S.M. PrevalLncia de anticorpos anti-*Neospora caninum* em cnes da área urbana do município de Monte Negro, Rondônia, Brasil. 12th Congresso Brasileiro de Parasitologia Veterinária, Rio de Janeiro, Brazil, 2002. Poster No. P-149.

Sreekumar, C., Hill, D.E., Fournet, V.M., Rosenthal, B.M., Lindsay, D.S., Dubey, J.P. Detection of Hammondia heydorni-like organisms and their differentiation from Neospora caninum using RAPD-PCR. 19th International Conference of the World Association for the Advancement of Veterinary Parasitology, "Old Dreams –New Visions: Veterinary Parasitology in the 21st Century" New Orleans 2003 Poster No. P-183

Lindsay, David S. and Dubey, J.P. *Sarcocystit neurona* (Protozoa: Apicomplexa): Description of sporocysts, excystation and early I vitro development. American Association of Veterinary Parasitologists 48th Annual Meeting Denver, Colorado 2003 Poster No. P-33

D.C., Dubey, J.P., Mansfield, L.S. Daily pyrantel tartrate does not prevent *Sarcocystis neurona* infection in horses. American Association of Veterinary Parasitologists 48th Annual Meeting Denver, Colorado 2003 Poster No. P-34

Dubey, J.P. Life cycle and transmission of a new species of *Bessnoitia* from domestic rabbits. American Association of Veterinary Parasitologists 48th Annual Meeting Denver, Colorado 2003 Poster No P-43

Almeria, S., Dawson, H., Dubey, J.P., Gasbarre, L. Cytokine gene expression at the materno-fetal interface in pregnant cows infected with *Neospora caninum*. American Association of Veterinary Parasitologists 48th Annual Meeting Denver, Colorado 2003 Poster No. P. 51

Bovine Functional Genomics Laboratory (BFGL)

Project Title: Improving Milk Quality by Reducing Mastitis in Dairy Cattle
CRIS Number: 1265-32000-065-00D
Scientists: Paape, M., Bannerman, D.
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Contact: Paape, M., Phone: (301) 504-8302, Fax: (301) 504-9498, mpaape@anri.barc.usda.gov

PROJECT OBJECTIVES:

Economic loss to the dairy industry due to mastitis can be reduced by enhancing the cow's natural defenses to bacterial infection of the mammary gland. Thus, means of improving the cow's natural defenses are urgently needed to minimize the use of antibiotics in preventing mammary gland infections. Widespread use of antibiotics has resulted in the development of resistant strains of bacteria which has necessitated periodic development of new more potent antibiotics. This poses the awesome possibility that the time is rapidly approaching when strains of bacteria will be produced that are resistant to any and all antibiotics. The use of antibiotics also poses the ever present problem of antibiotic contamination of the milk supply.

The bacteria causing the greatest loss to the dairy industry are Staphylococcus aureus and Gram-negative species. Staphylococcus aureus and gram-negative bacteria are ubiquitous in the environment and result in 85% of all cases of mastitis. More importantly once inside the gland S. aureus adhere to and penetrate tissues lining the gland to form abscesses that are impervious to antibiotics and host defense mechanisms. This results in chronic cases of mastitis that necessitate culling of valuable milking stock.

Infections by Gram-negative bacteria are especially debilitating if contracted soon after calving, usually resulting in death of the cow. Death is due to the failure of the udder to control the inflammation caused by endotoxin produced by the rapidly multiplying coliform organisms in the milk. It is estimated that 300,000 dairy cows either die or are culled from the herd because of acute coliform mastitis.

Current investigations are focused on determining the nature of S. aureus and Gram-negative bacteria virulence factors and means of shoring up the animal's defenses to combat them. Virulence factors under investigation are: 1) means of entrance of the bacteria into the gland, 2) mechanism(s) of avoiding host cellular and humoral defenses and 3) mechanism(s) of adherence and penetration into the tissue. Means of shoring up the animal's defenses to combat bacterial infection and neutralization of bacterial toxins under current investigation include: 1) strain variation in the various virulence factors, 2) strain distribution in the national herd, 3) incorporation of the predominant virulence factors in a vaccine(s), 4) methods of delivery of the vaccine(s), 5) immunomodulators to enhance the immune defenses, 6) nutritional factors effecting immune function and 7) cloning of genes responsible for neutralization of endotoxin.

OVERALL PROJECT ACCOMPLISHMENTS:
Staphylococcus aureus vaccines currently available are of limited effectiveness because they include only 40% of the serotypes identified in the national dairy herd. A trivalent S. aureus vaccine, that covers 100% of the serotypes in the national dairy herd, was used to immunize first calf heifers to prevent S. aureus mastitis and was used in conjunction with antibiotics to treat chronic S. aureus mastitis infections. The vaccine was 100% effective in protecting heifers from S. aureus infections and 70% effective in curing chronic infections, thus, preventing at the very beginning of the cow's productive life. This vaccine could significantly decrease dairyman's losses due to mastitis; to that end it is being tested in commercial herds in Michigan, Maryland and North Carolina to cure existing chronic S. aureus infections.

IMPACT:

TECHNOLOGY TRANSFER:
A phenolic teat dip was developed and shown to be effective in preventing new intramammary infections. This teat dip will be made available to dairymen. We have shown that treatment of beef cows at weaning by intramammary infusion of antibiotics will reduce incidence of mastitis and improve calf weaning weights. Adoption of this technology will increase beef cattlemen's profits.

Patents:
A patent, No. 0108.01, "Use of recombinant bovine CD14 in the treatment and prevention of coliform mastitis in dairy cows" was prepared and submitted to the Patent Office through the USDA Office of Technology Transfer.

PUBLICATIONS:

Lee, J.-W., Paape, M.J., Elsasser, T.H., Zhao, X. Elevated milk soluble CD14 in bovine mammary glands challenged with Escherichia coli Lipopolysaccharide, Journal of Dairy Science. 2003. v. 86. p.2382-2389.

Lee, J.-W., Paape, M.J., Zhao, X. Recombinent bovine soluble CD14 reduces severity of experimental Escherichia coli mastitis in mice. Veterinary Research. 2003. v. 34, p. 306-316.

Lee, J.-W., Paape, M.J., Elsasser, T.H., Zhao, X. Recombinant soluble CD14 reduces severity of mammary intection by Escherichia coli. Infection and Immunity. 2003. v. 71. p. 4034-4039

Bannerman, D.D., Goldblum, S.E. Mechanisms of bacterial lipopolysaccharide-induced endothelial apoptosis. American Journal of Physiology. 2003. v. 284. p.L899-L914.

Li, X., Tupper, J.C., Bannerman, D.D., Win, R.K., Rhodes, C.J., Harlan, J.M. Phosphoinositide 3 kinase mediates toll-like receptor 4-induced activation of NF-OB in endothelial cells. Infection and Immunity. 2003. v. 71, p. 4414-4420.

Erwert, R.D., Eiting, K.T, Tupper, J.C., Winn, R.K., Harlan, J.M., Bannerman,D.D. Shiga toxin induces decreased expression of the anti-apoptotic protein Mc1-1 concomitant with the onset of endothelial apoptosis. Microbial Pathogenesis. 2003. v. 35. p. 87-93.

Diez-Fraile, A., Meyer, E.,Paape, M.J., Burvenich, C. Analysis of selective mobilization of L-selectin and Mac-1 reservoirs in bovine neutrophils and eosinophils. Veterinary Research. 2003, v. 34. p. 57-70

Van Oostveldt, K., Paape, M.J., Dosogne, H., Burvenich, C. Effect of apoptosis on phagocytosis, respiratory burst and CD18 adhesion receptor expression of bovine neutrophils. Domestic Animal Endocrinology. 2003. v. 22. p. 37-50.

Lee, J.-W., Paape, M.J., Zhao, X. Soluble CD14 prevents intramammary infection by Escherichia coli. FASEB Journal. 2003. v. 17. p. A670.

Bovine Functional Genomics Laboratory (BFGL)

Project Title: Immunological and Genetic Basis of Resistance to Parasites of Cattle
CRIS Number: 1265-32000-072-00D
Scientists: Gasbarre, L., Zarlenga, D.
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PROJECT OBJECTIVES:

Gastrointestinal (GI) nematode infections cause major losses to the American cattle industry due to decreased productivity of infected animals. Current control procedures are based on the repeated administration of anthelmintic drugs to a large proportion of the cattle herd. Attempts to control parasites by pasture management are either ineffective, or are economically unfeasible. The alternative to these measures is to use the host immune system to control the intensity of parasite infection. Until recently there was little evidence that the bovine immune system was capable of limiting the magnitude of infection by GI nematodes. Studies in our laboratory have demonstrated that for most of the nematode species, even a short period of exposure is very effective in inducing the bovine immune system to reduce the number of parasites that become established in the host. The major exception to this is Ostertagia ostertagi, but even with this species, the immune system effectively reduces parasite transmission by reducing egg output by the female worms. These studies indicate that it is feasible to control nematode infections by using the host immune system. Before this method of control can be implemented a number of basic questions must be answered. At the present time nothing is known concerning the exact mechanism that results in resistance to infection. Similarly, there is a lack of information concerning the ability of Ostertagia to evade an immune response that appears to function very effectively against the other nematode species. Recently we have proven that host genetics play an important role in determining if individual cattle become immune or not, but as of this date little information concerning the genes involved is available. Finally, there is a complete lack of information available concerning the practical use of immunity-based systems in a modern production system. Information in these four areas is necessary for the integration of immunologically based control programs into a profitable and sustainable livestock management system.

Neosporosis is a parasitic disease primarily of dairy cattle that is caused by the protozoan parasite Neospora caninum. Although the parasite was first described as causing hind-limb paralysis in dogs, it is now recognized as a major cause of reproductive failure in cattle worldwide. This research program has the goal of understanding the immunological response to N. caninum so that ways of preventing neosporosis in cattle using vaccines or passive immunotherapy may be developed.

OVERALL PROJECTS ACCOMPLISHMENTS:
This project began in November of 2001, as such this is the second year of the project. Reference to the time line of the project indicates that : 1. QTL for parasite resistance have been identified, 2. the microarrays have been at least partially developed (i.e. more than 400 genes) and are being used to define resistant and susceptible cattle, and 3. cDNA libraries from Ostertagia have been prepared and are being screen for activity on the host immune system.

IMPACT:

TECHNOLOGY TRANSFER:
Presented talks to the Ambassador and Secretary of Agriculture of, Brazil on the identification of genes affecting disease resistance in cattle. Presented talk to selected international Veterinary students from the Cornell University Leadership program on modern concepts of parasite control.

Developed instructional aids for parasite control for beginning dairy and beef grazers .The PCR based diagnostic test continues to be used to assess parasite infection in local herds and validate the purity of local laboratory strains of parasites. Scientists from Montana have contacted us to evaluate the types of parasite infections present on local farms using this test.

PUBLICATIONS:

Wang, Y., Zarlenga, D.S., Paape, M.J., Dahl, G.E. Recombinant bovine CD-14 sensitizes the mammary gland to lipopolysaccharide. Journal of Veterinary Immunology and Immunopathology. 2002. v. 86. p. 115-124.

Gasbarre, L. C., Stout, W., Soder, K. Parasites. Beginning Grazier's Notebook, 2002, pp. 32-34.

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PROJECTS

Animal Health Research Unit, Clay Center, NE
- Genetic Predisposition of Livestock to Infection by Mucosal Pathogens
- Host Response and Antigenic Variation of Respiratory Viral Agents of Livestock

Animal Health Research

Project Title: Genetic Predisposition of Livestock to Infection by Mucosal Pathogens
CRIS Number: 5438-32000-023-00D
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Location: Clay Center, NE, U.S. Meat Animal Research Center, Animal Health Research
Contact: Heaton, M., Phone: (402) 762-4362, Fax: (402) 762-4375, heaton@email.marc.usda.gov

PROJECT OBJECTIVES:
Infectious diseases in livestock are a significant source of economic loss and represent a potential risk to human health. Removing animals with the highest risk of disease from the production cycle will lead to improved herd health and food safety. The ultimate goal of livestock genomics with regard to animal health is to read an animal's DNA sequence and estimate its risk of acquiring or maintaining infections. Our immediate aim is to evaluate natural genetic variation in diverse populations and identify alleles that predispose livestock to infectious diseases. Before this can be accomplished, two key issues need to be addressed: 1) the identification of nucleotide sequence variation in host genes that play a fundamental role in the infection process 2) the identification of gene haplotypes that are associated with susceptibility or resistance to infectious disease. Health traits of interest include: failure of passive transfer in cattle, ovine progressive pneumonia, prion disease in cattle sheep and deer, and bovine respiratory disease complex. Our research is designed to address these issues in commercial populations of livestock. As these objectives are achieved for selected candidate genes, new information and technology will be developed that will facilitate reading an animal's DNA sequence for estimating its risk of acquiring or maintaining infections.

OVERALL PROJECT ACCOMPLISHMENTS:
Discovery of genetic markers associated with failure of passive transfer (FPT) in new born calves. FPT is a condition in which neonates do not acquire protective serum levels of maternal antibodies from their mothers via colostrum. Calves with FPT are at significant risk for morbidity and mortality from infectious disease. The neonatal receptor for the Fc portion of immunoglobin, FcRn, is a heterodimer of MHC-1 alpha-chain homolog (encoded by the *FCGRT* locus) and beta-2-microglobulin (encoded by the *B2M* locus). This receptor functions both in uptake of IgG1 from the neonatal intestine as well as regulating the concentration of immunoglobin in the colostrum. Nucleotide sequence diversity was identified in these gene loci in U.S. beef cattle and evaluated for correlation with FPT in neonatal calves. Specific alleles of both *FCGRT* and *B2M* genes were found to have significant correlation with low serum levels of IgG (odds ratios of 3.8 and 10.6, respectively). One combination of alleles was found only in calves that failed passive transfer. Moreover, the two markers together accounted for 50% of the FPT cases in the study. Thus, these alleles represent potentially useful DNA markers for managing genetic risk of FPT.

Identification of new prion gene variation in cattle, sheep, and deer and development of accurate DNA tests for scrapie resistance. Prion gene (*PRNP*) variation has been well correlated with susceptibility to TSEs in humans, sheep, and rodents. Similar correlations have not yet been

reported in cattle or deer; however characterization of *PRNP* nucleotide diversity in those species is incomplete. We hypothesized that there is unrecognized and important nucleotide variation in the prion genes of cattle and deer that may influence TSE susceptibility. To test this, the complete *PRNP* coding region and a portion of *PRNP* promoter were sequenced in diverse groups of U.S. beef cattle, white-tailed and mule deer, and U.S. sheep. Sequence comparisons identified a total of 54 new polymorphisms. This allowed us to design the most accurate and efficient DNA tests for scrapie resistance in sheep.

Developed the first set of bovine single nucleotide polymorphism (SNP) markers for animal identification, parentage, and traceback in cattle. To identify SNP markers that would be appropriate for genetic testing in commercial populations, we designed and assembled two panels of genomic DNA: one from 96 sires from 17 breeds that represent the breadth and composition of cattle germ plasm used in the U.S. beef industry, and another from a panel of 192 purebred American Angus from seven herds in four Midwestern states. These two panels were used to identify and test SNP markers for determining the genetic identity and kinship of an animal. We identified a select set of 32 highly informative SNP markers distributed among 18 autosomes and both sex chromosomes. These DNA markers were used in confirming the country of origin for the Holstein cow in Washington State that tested positive for BSE on December 23, 2003. There are now more than 45 SNPs in this collection.

Development of essential tools and information for large-scale automated SNP discovery and genotyping in beef cattle. Dissection of complex traits in cattle from commercial populations will require the ability to genotype large numbers of animals and an abundant supply of validated DNA markers specific for candidate genes. SNP markers are a potential resource for analyzing complex traits because of their high frequency, low mutation rates, and amenability to automated analysis. We reported more than 45 SNPs markers and 40 haplotype structures in 9 bovine cytokine genes. Moreover, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was demonstrated as a leading technology for livestock SNP genotyping.

Expression, genetic mapping, and haplotyping of bovine genes induced by *Escherichia coli* O157H7. A potential method for reducing the impact of infectious disease in livestock is to increase the host genetic resistance to infection. The first line of defense against enteric infections are the epithelial mucosa and the immune mechanisms activated by contact with bacterial envelopes. This contact initiates responses leading to local inflamation and the eventual clearance of infection. We identified and mapped two bovine genes that were differentially expressed in bovine epithelial cells during an inflammatory response to *E. coli* O157H7. These genes, interleukin (*IL*) *8* and epithelial cell inflammatory protein (*ECIP*)-*1*, were both members of the C-X-C chemokine family, a group of proteins that play a pivotal role in mobilizing inflammatory cells in response to infectious challenges. The *ECIP1* gene had not previously been identified in any species and is thought to represent a novel chemokine induced by *E. coli* O157:H7. In subsequent research, we defined the *IL8* haplotype structure in U.S. beef cattle and estimated the ancestral allele.

IMPACT:
An important application of disease research and DNA marker technology is the identification of individuals with the highest risk for infectious disease and traceback of diseased animals. Our research has provided more than 300 single nucleotide polymorphisms (SNP) markers and assays to distinguish between nucleotide sequence variants of candidate genes in popular breeds of U.S. cattle, sheep, and deer. These DNA markers have been placed in the public domain and transferred to more than 40 companies and institutions in more than 19 countries. The markers have facilitated the identification of genotype/phenotype associations and ultimately allowed prediction of an individual's genetic predisposition for infection from its DNA sequence. All of these markers are available for use without restriction.

During the Washington State BSE, a collection of these DNA markers were used to confirm the Canadian origin of the BSE index case. This DNA testing result coincided with the recovery of live cattle markets. During the market drop, losses due to BSE was estimated at $10 M per day.

FPT is a serious problem in beef and dairy cattle and has both environmental and genetic influences. The two publications resulting from this research represent the first use of SNP markers and genetic epidemiology in cattle health traits and provide a road map for future execution of related studies.

TECHNOLOGY TRANSFER:
Bovine SNP markers and assay designs for determining identity and parentage have been transferred to several commercial entities for general use in the cattle industry. This has been accomplished through peer-reviewed scientific publications, deposition of information in GenBank at NCBI, a Specific Cooperative Agreement (SCA), and a Cooperative Research and Development Agreement (CRADA). At least two privately held companies have already placed their version of these cattle DNA tests in the hands of their customers. At least one large commercial ranch in Texas has already made use of these tests for animal ID and parentage. At least four European and Asian countries have requested the publically available SNP markers and assay design.

SNP markers and assay design for prions genes in U.S. sheep, cattle, and deer have also been transferred to several commercial genotyping companies and diagnostic labs for general use. Our sheep prion genoytping assays are being used commercially in the national scrapie eradication program. The cattle and deer information will likely be used in the epidemiology of bovine spongiform encephalopathy (BSE) and chronic wasting disease (CWD), respectively.

PUBLICATIONS:

Clawson, M. L., M. P. Heaton, C. G. Chitko-McKown, J. M. Fox, T. P. L. Smith, W. M. Snelling, J. W. Keele, and W. W. Laegreid. 2004. Male-specific SRY and ZFY haplotypes in U.S. beef cattle. *Animal Genetics* 35:246-249.

Clawson, M. L., M. P. Heaton, C. G. Chitko-McKown, J. M. Fox, T. P. L. Smith, W. M. Snelling, J. W. Keele, and W. W. Laegreid. 2004. Beta-2-microglobulin haplotypes in U.S. beef cattle and association with failure of passive transfer in newborn calves. *Mamm. Genome.* 15:227-236.

Heaton MP, Leymaster KA, Freking BA, Hawk DA, Smith TPL, Keele JW, Snelling WM, Fox JM, Chitko-McKown CG, Laegreid WW. 2003. Prion gene sequence variation within diverse groups of U.S. sheep, beef cattle, and deer. *Mamm. Genome* 14:765-777.

Laegreid WW, Heaton MP, Keen JE, Grosse WM, Chitko-McKown CG, Smith TPL, Keele JW, Bennett GL, Besser TE. 2002. Association of bovine neonatal Fc receptor alpha-chain gene (*FCGRT*) haplotypes with serum IgG concentration in newborn calves. *Mamm. Genome* 13:1-7.

M. P. Heaton, G. P. Harhay, G. L. Bennett, R. T. Stone, W. M Grosse, E. Casas, J. W. Keele, T. P.L. Smith, C. G. Chitko-McKown, W. W. Laegreid. 2002. Selection and use of SNP markers for identification and paternity analysis in U.S. beef cattle. *Mamm. Genome* 13:272-281.

Page, B. T., E. Casas, M. P. Heaton, N. G. Cullen, D. L. Hyndman, C. A. Morris, A. M. Crawford, T. L. Wheeler, M. Koohmaraie, J. W. Keele, and T. P. L. Smith. 2002. Evaluation of single-necleotide polymorphisms in *CAPN1* for association with meat tenderness in cattle. J. Anim. Sci. 80:3077-3085.

Heaton, M.P., C.G. McKown, W.M. Grosse, J.E. Keen, and W.W. Laegreid. 2001. Interleukin-8 haplotype structure from nucleotide sequence variation in commercial populations of US cattle. *Mamm. Genome*. 12:219-225.

Smith, T.P.L., W.M. Grosse, B.A. Freking, A.J. Roberts, R.T. Stone, E. Casas, J.E. Wray, J. White, J. Cho, S.C. Fahrenkrug, G.A. Bennett, M. P. Heaton, W.W. Laegreid, G.A. Rohrer, C.G. Chitko-McKown, G. Pertea, I. Holt, S. Karamycheva, F. Liang, J. Quackenbush, J.W. Keele. 2001. Sequence evaluation of four pooled-tissue normalized bovine cDNA libraries and construction of a gene index for cattle. *Genome Res.* 11:626-630.

Heaton, M.P., W.M. Grosse, S.M. Kappes, J.W. Keele, C.G. Chitko-McKown, L.V. Cundiff, A. Braun, D.P. Little and W.W. Laegreid. 2001. Estimation of DNA sequence diversity in bovine cytokine genes. *Mamm. Genome*. 12:32-37.

Animal Health Research

Project Title: Host Response and Antigenic Variation of Respiratory Viral Agents of Livestock
CRIS Number: 5438-32000-024-00D
Scientists: Fox, J., Heaton, M., Vacant
Location: Clay Center, NE, U.S. Meat Animal Research Center, Animal Health Research
Contact: Fox, J., Phone: (402) 762-4392, Fax: (402) 762-4375, fox@email.marc.usda.gov

PROJECT OBJECTIVES:

Respiratory viral infections caused by arteriviruses and paramyxoviruses result in significant economic losses in the livestock industry worldwide. For example, Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) infections within the U.S. account for up to 15-20% of the economic losses yearly in the swine industry. Such economic losses from respiratory viral infections result from direct mortality, reduced production efficiency, and treatment/prevention costs to combat infection. In addition, many of the respiratory bacterial infections present in livestock result from impairment of pulmonary defense mechanisms by respiratory viral infections. Bacterial pneumonias can result in severe losses in production, be costly to treat, and, in some cases, persist for long periods of time. Reducing the incidence of respiratory viral infections will not only improve production efficiency normally lost to the viral diseases, but will also significantly reduce the incidence of bacterial pneumonia and losses that result from these infections. There is limited information known about the host cell response to infection by these viral agents. Examining this response is necessary to gain a better understanding of the dynamics of the virus-to-cell interactions. The development of better vaccines will be dependent upon a more comprehensive understanding of the virus-to-host interactions and how they effect disease expression.

OVERALL PROJECT ACCOMPLISHMENTS:

The development of the MARC-145 cells line is perhaps the single largest accomplishment of this project. This cell line is currently utilized world-wide for the in vitro propagation of PRRSV. In addition, significant progress has been made on analysis of PRRSV in populations of swine. Recombinant antigens have been developed and immunity to individual PRRSV proteins evaluated in sera from naturally infected swine from around the country. Furthermore, these antigens have been used to investigate the differences in immune responses over time in swine persistently infected with PRRSV. In combination with sequence analysis of a number of PRRSV isolates, this work will result in an understanding of the host-virus interaction in the field. Preliminary data from these studies has indicated which viral genes are responsible for induction of a protective immune response to PRRSV and the relevant genes cloned into vectors for use as vaccine candidates. In addition, we have demonstrated that antibody alone is sufficient to protect against PRRSv infection in the pregnant sow model. Using molecular genomic approaches we have examined the host cell response to infection. Over 100 genes have been identified whose mRNA abundance is altered as a result of PRRSV infection. Interestingly,

as important as those responsive genes identified, are a number of genes that are not responsive to PRRSV infection but would be expected to respond. Within these genes are those belonging to the Type I interferon response to infection. We have shown that PRRSV specifically interferes with the Type I interferon response resulting in little to no change in Type I interferon levels produced by infected cells. This may have broad implications on the delayed immune response to PRRSV infection. Further understanding and characterization of this phenomenon may lead to the design of vaccine delivery systems that will better trigger the innate immune response.

IMPACT:

TECHNOLOGY TRANSFER:
PRRSV sequence data has been submitted to GenBank for public access. Cultures of MARC-145 cells were distributed to several investigators.

PUBLICATIONS:

Allende R., Laegreid W.W., Kutish G.F., Galeota J.A., Wills R.W., Osorio F.A. (2000) Porcine reproductive and respiratory syndrome virus: description of persistence in individual pigs upon experimental infection. J Virol 74: 10834-7.

Allende R., Kutish G.F., Laegreid W.W., Lu Z., Lewis T.L., Rock D.L., Friesen J., Galeota J.A., Doster A.R., Osorio FA. (2000) Mutations in the genome of porcine reproductive and respiratory syndrome virus responsible for the attenuation phenotype. Arch Virol 145: 1149-61.

Osorio F.A., Galeota J.A., Nelson E., Brodersen B., Doster A., Wills R., Zuckermann F., Laegreid W.W. (2002) Passive transfer of virus-specific antibodies confers protection against reproductive failure induced by a virulent strain of porcine reproductive and respiratory syndrome virus and establishes sterilizing immunity. Virology 302: 9-20.

Miller, L. C., Laegreid, W. W., Bono, J. L., Chitko-McKown, C. G., and Fox, J. M. (2004) Interferon Type I response in porcine reproductive and respiratory syndrome virus-infected MARC-145 cells. Arch Virol (*in press*).

Miller, L. C. and Fox, J. M. (2004) Apoptosis and porcine reproductive and respiratory syndrome virus. Vet. Immunol. and Immunopath. (*in press*).

Arthropod-Borne Animal Diseases Research (ABADRL)
Laramie, WY

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PROJECTS

Arthropod-Borne Animal Diseases Research

- Vector Competence and Protection of U.S. Livestock and Wildlife from Arthropod-Borne Diseases
- Molecular Biology and Pathogenesis of Arboviruses
- Development and Validation of Rapid Field Deployable Genetic Tests for Vesicular Stomatitis Virus

Arthropod-Borne Animal Diseases Research Projects

Project Title: Vector Competence and Protection of U.S. Livestock and Wildlife from Arthropod-Borne Diseases
CRIS Number: 5410-32000-010-00D
Scientists: Schmidtmann, E., Drolet, B., Mecham, J., Wilson, W., Letchworth III, G., Mayer, R.
Location: Laramie, WY, Arthropod-Borne Animal Diseases Research
Contact: Schmidtmann, E., Phone: (307) 766-3630, Fax: (307) 766-3500, eschm@uwyo.edu

PROJECT OBJECTIVES:
Project 5410-3200-010-00D.

Certain blood-feeding insects efficiently transmit several indigenous and exotic disease-causing viruses that adversely affect the economy of the U.S. livestock industry. The emergence of West Nile virus (WNV) in the U.S. clearly demonstrates that exotic insect-transmitted pathogens can be imported and become established. The intentional or un-intentional importation of mosquito-transmitted Rift Valley Fever Virus would cause far more catastrophic human and animal loses than did WNV. Therefore, the overall goal of this project is to develop knowledge and tools to protect US livestock from current and potential insect-transmitted pathogens.

Bluetongue, epizootic hemorrhagic disease, vesicular stomatitis, and West Nile viruses account for acute and chronic production-related losses among U.S. livestock. Of greater economic concern in the global economy of the 21st Century, these insect-transmitted diseases trigger non-tariff trade barrier restrictions that limit export sales of U.S. livestock and their germplasm. The key role that insects play as vectors of viral pathogens is a product of: 1) insect blood-feeding on host animals as the primary mode of arbovirus transmission in nature; 2) insects as a source of virus amplification and maintenance; and 3) the seasonal incidence and geographical distribution of risk for host exposure.

Research conducted under this CRIS project will benefit U.S. agriculture by providing a better understanding of: 1) the molecular mechanisms that regulate the competence of insects that transmit pathogenic viruses, and 2) the environmental factors that determine vector population distribution and abundance. This research opportunity is optimised by the availability of recent molecular and computer-aided data processing and methods that provide insights and capabilities that were previously unavailable. Information developed through this research will benefit U.S. livestock commodities by strengthening the rural economy, providing a continued supply of inexpensive, wholesome food products for the American consumer, as well as promoting the profitability and international competitiveness of livestock products. Proposed research will support the development of novel strategies for controlling the vector, or its vector competence, as well as provide a basis for scientifically-based domestic and international trade regulations that protect U.S. livestock from the continuous threat of indigenous, exotic, and emerging insect-borne diseases.

OVERALL PROJECT ACCOMPLISHMENTS:
On the basis of ABADRL field and laboratory research, the biting midge, *Culicoides sonorensis*, has been implicated as the primary, proven vector of bluetongue viruses in the U. S.

Evidence developed by the ABADRL also established that the northeast U.S. is bluetongue-free because it is outside the range of *C. sonorensis*. The population genetics of the *C. variipennis* complex in North America was investigated, and resulted in a taxonomic revision (i.e. current subspecies were elevated to three species). It was demonstrated that levels and types of dissolved salts in larval habitats differ among aquatic habitats occupied by the three species of the complex. This finding opens the way for large-scale mapping and prediction of *C. sonorensis* using computer-based GIS methods. The demonstration of bluetongue-free regions of the U.S. has resulted in a relaxation of export regulations, thus improving the competitiveness of U.S. livestock and livestock germplasm in world markets. Research that utilizes the genetic markers and cloned genes produced by the ABADRL will increase our understanding at the molecular level of the vector competence of *C. sonorensis* for bluetongue and epizootic hemorrhagic disease viruses. Reducing the impact of existing arthropod-borne pathogens and protecting U.S. animal agriculture from new and emerging animal pathogens is dependent on this research.

Recent ABADRL research identified a non-hematophagous insect (grasshopper) as a potential source of infection and maintenance reservoir of vesicular stomatitis virus. This study demonstrated that VSV replicates in grasshoppers and can spread between grasshoppers and cattle, which may lead to better understanding the epidemiology of sporadic outbreaks of VSV among western livestock. Analysis of the distribution of the sand fly, *Lutzomyia apache*, in the mid-Rio Grande River Valley, New Mexico, using geographic information system methods indicates a potential role for this sand fly in the epidemiology of VS viruses in the southwestern U.S.

IMPACT:
ABADRL research has established the northeast US as a bluetongue-free region, resulting in a relaxation of export regulations, thus improving the competitiveness of U.S. livestock and livestock germplasm in world markets.

The ABADRL has established an Insect Vector Genomics program that has increased understanding of the genetic components that affect the ability of insects to be disease vectors (vector capacity) and will help predict the risk of pathogen transmission.

ABADRL's investigations of the role of the potential of VSV transmission by several insect species have suggested new paradigms for the epidemiology of VSV.

TECHNOLOGY TRANSFER:
Technology transfer may be expected of the VSV-specific IHC assay in the future. If a VSV outbreak occurs, the clinical similarity between VSV and foot and mouth disease will require rapid virus specific assays that can differentiate the two viruses. In some cases, the incriminating positive cell culture results will have already been fixed and stained, destroying the live sample. The IHC assay can confirm CPE as VSV-specific after live samples have been destroyed, and therefore will be a valuable tool. The assay is ready for use in VSV-infected cell cultures. The

use of synthetic pyrethroid insecticides as repellents for application to livestock has been transferred via verbal and written communication to horse owners, veterinarians and entomologists concerned about protecting animals from West Nile virus. Control methods for larval stages of *C. sonorensis* have been transferred to white-tailed deer farmers who have used the recommendations to prevent annual deer die-offs from biting midge-transmitted viruses. The results of the multi-state Bluetongue Surveillance Pilot study have been reported to participating producers and veterinary associations; this information will be useful in the formulation of regulations concerning export of U.S. cattle to bluetongue-free countries. Information concerning the presence and seasonal abundance of *Culex tarsalis*, the primary vector of West Nile virus in the western U.S., has been transferred to state and county level personnel concerned with public health and mosquito control, as well as coal bed methane gas developers whose well drilling activities produce impoundments that support mosquito larval development.

Cooperative Research Projects have been initiated with the Department of Molecular Biology, University of Wyoming; and the Department of Microbiology, Immunology and Pathology, Colorado State University.

ABSTRACTS:

Nunamaker, R.A., Lockwood, J.A., Stith, C.E., Campbell, C.L., Schell, S.P., Drolet, B.S., Wilson, W.C., White, D.M. and Letchworth. G.J. Are grasshoppers a source for vesicular stomatitis virus? The ESA Annual Meeting: An Entomological Odyssey of ESA San Diego, CA December, 2001.

Lockwood, J.A., Nunamaker, R.A., Stith, C. E., Campbell, C.L., Schell, S.P., Drolet, B.S., Wilson, W.C., White, D.M. and Letchworth. G.J. Grasshoppers as vectors and reservoirs of vesicular stomatitis. The ESA Annual Meeting: An Entomological Odyssey of ESA, November, 2002.

Campbell, C.L., McNulty, M.J., Letchworth, G.J. and Wilson, W.C. Molecular Investigations of virus/vector interactions. OIE Symposium on Bluetongue Virus. Taormina, Italy, 2003. Abs. #B37, pg 77. October, 2004

Letchworth, G.J., Nunamaker, R.A., Stith, C.E., Campbell, C.L., Schell, S.P., Drolet, B.S., Wilson, W.C. and White, D.M. Grasshoppers as vectors and reservoirs of vesicular stomatitis. Poster. Rocky Mountain Virology Club Meeting, Pingree Park, CO, September, 2003.

Campbell, C.L., VanDyke, K.A., Mummey, D.L., McNulty, M.J., Letchworth, G.J. and Wilson, W.C. Functional genomics of vector competence in the arbovirus vector, *Culicoides.* Keystone Symposium, "Genetic Manipulations of Insects", February, 2004, Taos, NM.

Campbell, C.L., Vandyke, K.A., Letchworth, G.J., Drolet, B.S. and Wilson, W.C. *Culicoides* gene discovery project reveals putative pharmacological factors in midge saliva. Entomological Society of America annual meeting, Salt Lake City, UT, November, 2004.

PUBLICATIONS:

Campbell, C.L. and Wilson, W.C. 2002. Differentially expressed midgut transcripts in *Culicoides sonorensis* (Diptera: Ceratopogonidae) following Orbivirus (Reoviridae) oral feeding. Insect Mol. Biol. 11:595-604.

O'Toole, D., Perez de Leon, A.A., Hearne, C., McHolland, L., Yun, L. Tabachnick. W.J. 2003. Papular dermatitis induced in guinea pigs by the biting midge *Culicoides sonorensis* (Diptera: Ceratopogonidae). J.Vet. Diagn. Invest. 15:67-71.

Drolet, B.S., Campbell, C.L. and Mecham, J.O. 2003. Protect yourself and your sample: Processing arbovirus infected biting midges for viral detection assays and differential expression studies. In, J. Richmond (ed.), Anthology of Biosafety: Arthropod-Borne Diseases, Chapter 5, pp. 53-62.

Nunamaker, R.A., Lockwood, J.A., Stith, C.E., Campbell, C.L., Schell, S.P., Drolet, B.S., Wilson, W.C., White, D.M. and Letchworth, G.J. 2003. Grasshoppers can serve as reservoirs and vectors of vesicular stomatitis virus. J. Med. Ent. 40:957-963.

Campbell, C.L., Mummey, D.L., Schmidtmann, E.T. and Wilson. W.C. 2004. Culture-independent analysis of midgut microbiota in the arbovirus vector *Culicoides sonorensis* (Diptera: Ceratopogonidae). J. Med. Ent. 41:340-348.

Naugle, D.E., Walker, B.L., Aldridge, C.L., Cornish, T.E., Moynahan, B.J., Holloran, M.J., Brown, K., Johnson, G.D., Schmidtmann, E.T., Mayer, R.T., Kato, C.Y., Matchett, R.R., Christiansen, T.J., Cook, W.E., Creekmore, T., Boyce, M.S., Falise, R.D. and Rinkes. T.E. 2004. West Nile virus: Pending crisis for greater sage grouse. Ecol. Letters 7: 704-713.

Campbell, C.L., McNulty, M. J., Letchworth, G. J. and Wilson, W.C. 2004. Molecular investigations of virus/vector interactions. IV International Conference on Bluetongue. Taormina, Italy, (In Press).

Dargatz, D. Akin, G., Green, M., Herrero, M., Holland, S., Kane, A., Knowles, K., McElwain, T., Moser, K., Ostlund, E., Parker, M., Schmidtmann, E., Seitzinger, A. Schuler, L., Stevens, G., Tesar, L., White, L., Williams, L., Wineland, N., and Walton, T.E. 2004. Bluetongue surveillance methods in the United States. IV International Conference on Bluetongue, Taormina, Italy, (in press).

Herrero, M.V., Yarnell, W. K. and Schmidtmann, E. T. 2004. Landscape associations of the sand fly, *Lutzomyia apache* (Dipetera: Psychodidae) in the southwestern United States: a Geographic Information System analysis. J. Vect. Ecol. (in press).

Mullens, B.A., Gerry, A.C., Lysyk, T.J. and Schmidtmann, E.T. 2004. Environmental effects on vector competence and virogenesis of bluetongue virus in *Culicoides*: interpreting laboratory data in a field context. IV International Conference on Bluetongue, Taormina, Italy, (in press).

Arthropod-Borne Animal Diseases Research

Project Title: Molecular Biology and Pathogenesis of Arboviruses
CRIS Number: 5410-32000-011-00D
Scientists: Mecham, J., Drolet, B., Wilson, W. Letchworth III, G., Schmidtmann, E., Mayer, R.
Location: Laramie, WY, Arthropod-Borne Animal Diseases Research
Contact: Mecham, J., Phone: (307) 766-3620, Fax: (307) 766-3500, jmecham@uwyo.edu

PROJECT OBJECTIVES:
Project 5410-32000-011-00D.

Arthropod-borne viruses (arboviruses) are important pathogens of livestock and have a significant impact on the U.S. livestock industry. These viruses have a complex life cycle and infect both vertebrate and invertebrate hosts. The proposed research will delineate viral and host characteristics important in the infection cycle, epidemiology, and pathogenesis of these viruses. This information will provide the basis for the development of more effective diagnostics and novel control strategies. The Arthropod-Borne Animal Diseases Research Laboratory is developing the tools necessary to diagnose, control and asses risk of insect-transmitted pathogens of current concern to US livestock industry; however, the technology developed could be rapidly adapted to respond to emerging disease concerns.

Specifically, the differences in replication of BTV and EHDV in vertebrate and invertebrate hosts are not well understood. Research to determine the mechanisms of replication of these viruses in these different hosts may reveal weaknesses that can be used to develop unique control strategies or improved vaccines. BTV and EHDV undergo genetic mutation and reassortment to give rise to new viral strains. Investigating the genetic relationships and evolution of these orbiviruses will provide more information on the epidemiology of disease and assist with development of improved diagnostics and control strategies. Such information will aid the development of rapid, field deployable diagnostic tests for the detection of viral antibody, viral particles, viral antigens, and viral nucleic acids. The pathogenesis of epizootic hemorrhagic disease virus (EHDV) infection in cattle is not well understood. Research will determine if the difference in EHDV virulence between cattle and deer is related to endothelial cell tropism and the effect on the fetus of EHDV infection in pregnant cattle. Effective control of infections caused by BTV and EHDV requires sensitive and specific diagnostics. Epizootics caused by West Nile and vesicular stomatitis viruses have resulted in significant morbidity and mortality in the equine and bovine industries. At present there is no vaccine for VSV and only a provisionally licensed killed vaccine for WNV. The development and evaluation of vaccines for both these viruses is proposed. Monitoring of West Nile Virus infected mosquitoes is necessary for effective vector control. Current WNV surveillance measures are time consuming and costly due to manual mosquito sorting and a two-step diagnostic procedure (RNA isolation and RT-PCR). This laboratory is developing a diagnostic procedure, based on RNA capture that will eliminate the above steps and can be coupled with mosquito species-specific markers. The dual determination of mosquito species and presence or absence of WNV should expedite WNV surveillance and also serve as a model system for other arboviruses.

OVERALL PROJECT ACCOMPLISHMENTS:
Project 5410-32000-011-00D.

ABADRL research has laid the foundation for the development of diagnostic tests and control strategies for arboviruses. Prior research yielded information on the molecular characteristics of BTV, EHDV, WNV and VSV and a greater understanding of the evolutionary relationships of members of these virus groups, viral characteristics associated with virulence and pathogenesis, replication in both invertebrate vectors and vertebrate hosts, and the immune response of the vertebrate host following viral infection. Genetic studies produced epidemiological information relating to the evolution and movement of the BTV and EHDV serogroups. Genetic and protein characterization of BTV and EHDV led to the identification and characterization of the BTV protein (VP7) that is important in infection of the *Culicoides* vector and the development of both gene-based and antibody-based diagnostic procedures with increased sensitivity and specificity. The function of different viral proteins in neutralization, virulence and pathogenesis was investigated and described. Genes from BTV, EHDV, VSV, and WNV were cloned into expression vectors capable of infecting various cells. Methods were developed to increase the expression of specific viral proteins. These vectors and the expressed proteins will be used in DNA vaccine trials, as recombinant vaccines as a source of antigen for the development of diagnostic assays, and as tools to analyze steps in viral replication in both vertebrate and invertebrate hosts. A number of cell lines were developed and identified for studying BTV, EHDV and VSV replication. *Culicoides sonorensis* cell lines from colony and field populations were developed and characterized. Vector cell lines are essential tools in dissection of vector-virus interactions. Cell lines were identified that do not support productive infection of BTV, and are being used to elucidate the steps of virus replication in vertebrate cells. Genes from an equine isolate of WNV have been cloned and are being used as standards for the development of diagnostic reagents and for recombinant vaccines. The ABADRL participated in a collaborative study with Colorado State University and the Centers for Disease Control in Fort Collins that indicated there is little risk that horses infected with West Nile virus can transmit virus back to mosquitoes.

The role of *C. sonorensis* as a vector of VSV has been examined in the laboratory using procedures, such as immunohistochemistry, which show that VSV passes through both the midgut and salivary barriers, and thus infected insects may be competent biologic vectors. The presence of VSV in the eggs suggests that VSV may transfer vertically from females to their offspring. Both RT-PCR and *in situ* hybridization assays were used to confirm VSV replication in the virus fed insects.

The role of *Culicoides* saliva in arboviral infections is not known. ABADRL scientists initiated experiments to determine whether *Culicoides* saliva had an effect on the ability of VSV to establish an infection in horse skin cells. Viral infection increased with increasing amounts of insect saliva, potentiating the infection.

IMPACT:
ABADRL has developed several immunological or genetic based diagnostic tests or modifications of these assays that are used worldwide. Some of these assays are available commercially. These test are rapid, sensitive and have been used to set the diagnostic standards for international trade requirements.

Information produced by the ABADRL on the pathogensis of arboviral diseases has been used in risk assessment and in international trade negotiations.

ABADRL scientists are sought nationally and internationally for scientific advice on diagnosis, control and monitoring of emerging and indigenous insect-transmitted pathogens.

TECHNOLOGY TRANSFER (Recent):
Antibodies, PCR reagents and diagnostic protocols have been provided to researchers and diagnosticians in the U.S. and in several foreign countries.

A competitive enzyme-linked immunosorbent assay for detection of antibody to epizootic hemorrhagic disease virus developed at the ABADRL has been transferred to the NVSL, Ames, IA for further testing and validation.

Culicoides sonorensis cell lines were distributed to researchers in the U.S. for isolation and characterization of arboviruses.

Cooperative agreements were established with the Wyoming Agriculture Department, the Wyoming Game and Fish Department, the Wyoming Public Health Department were established, the University of Wyoming School of Pharmacy, the University of Wyoming GIS center, and with Dow Agrosciences.

Collaborations continued or were developed with the University of Wyoming, the University of California, Davis, and with Louisiana State University.

Reviews of research at the ABADRL and modern detection methods for bluetongue, epizootic hemorrhagic and vesicular stomatitis disease viruses was presented to National Cattlemen, Wyoming Stockgrowers Association/Wyoming Woolgrowers Association Wyoming Wool Growers representatives, National Quarantine Instititute, Qindao China, and the U.S. Armed Forces Research Institute for Infectious Diseases. Most of this technology is available to industry partners and new methods are currently being validated.

Research information was presented at meetings of the OIE Symposium for bluetongue virus, U.S. Animal Health Association Meeting, American Society for Virology Meeting, Keystone symposium, Colorado State University's Infectious Diseases Colloquium and the Rocky Mountain Virology Club Meeting.

ABSTRACTS:

Drolet, B.S. *Culicoides sonorensis* as a potential biological vector for vesicular stomatitis virus. American Society for Virology. Madison, Wisconsin. July, 2001.

Mecham, J.O. and Wilson, W.C. Application of baculovirus expressed antigens in enzyme-linked immunosorbent assays for detection of antibody to bluetongue and epizootic hemorrhagic disease viruses. American Association of Veterinary Laboratory Diagnosticians, Hershey, Pennsylvania, 2001, pp. 20.

Mecham, J.O. Neutralization of bluetongue virus in invertebrate cells. American Society for Virology, Madison, Wisconsin, 2001, Abs. #P23-1.

Drolet, B.S. Vesicular stomatitis virus, a re-emerging disease. Rocky Mountain Virology Club Meeting, September, 2002.

Drolet, B.S. and Lam, V. Vesicular stomatitis virus potentiation by *Culicoides* salivary gland homogenates. Poster. Rocky Mountain Virology Club Meeting, September, 2002.

Wilson, W.C., Xia, D., Larson, M.D., Hadfield, T.L. and Mecham, J.O. Rapid detection of arboviral RNA using fluorescent amplification assays. American Society for Virology, Lexington, Kentucky, 2002, Abs. #P11-2.

Mecham, J.O. Evidence for reassortment in the natural history of bluetongue virus serotype 2. American Society for Virology, Davis, CA, 2003, W39-9.

Balasuriya, U.B.R., Nadler, S.A., Wilson, W.C., Smythe, A.B., De Santis, P., Zhang, N., MacLachlan, N.J. Global isolates of bluetongue virus segregate into region-specific topotypes based on phylogenetic analysis of their NS3 gene. OIE Symposium on Bluetongue Virus. Taormina, Italy, 2003. Abs. # C1, pg 88. October, 2004.

Campbell, C.L., Mummey, D.L., VanDyke, K.A., McNulty, M.J., Letchworth, G.J. and Wilson. W.C. Functional genomics of vector competence in the arbovirus vector *Culicoides* spp. Keystone Symposium. February, 2004.

White, D.M., Wilson, W.C., Blair, C.D., and Beaty, B.J. Possible overwintering mechanism of bluetongue virus in vectors. OIE Symposium on Bluetongue Virus. Taormina, Italy, 2003. pg 40. October, 2004.

White, D.M. and Mecham, J.O. Possibility of bluetongue virus in bovine skin. OIE Symposium on Bluetongue Virus. Taormina, Italy, 2003. Abs. #C9, pg 96. October, 2004.

Wilson, W.C., Stallknecht, D.E. and Mecham, J.O. Field-deployable real-time PCR detection of bluetongue and epizootic hemorrhagic disease viral RNA. OIE Symposium on Bluetongue Virus. Taormina, Italy, 2003. Abs. #D14, pg 117. October, 2004.

Drolet, B.S. and Lam, V. Potentiation of vesicular stomatitis virus infection of equine cells by *Culicoides* salivary gland homogenates. Poster. 22nd Annual American Society for Virology Meeting, Davis, CA, July, 2003.

Drolet, B.S. and Wilson, W.C. Infection pathways of vesicular stomatitis virus in the biting midge, Culicoides sonorensis. CSU Research Colloquium Series: Infectious Diseases Meeting, Fort Collins, CO, April, 2004.

Drolet, B.S., Campbell, C.L., Stuart, M.A. and Wilson, W.C. Replication of vesicular stomatitis virus in the biting midge, *Culicoides sonorensis*. Poster. 23rd Annual American Society for Virology Meeting, Montreal Canada, July, 2004. Abs. #P13-13.

Mecham, J.O. Detection and quantitation of bluetongue virus in insect cell culture. American Society of Virology, Montreal, Canada, 2004, Abs. # P35-7.

Drolet, B.S., Campbell, C.L., Stuart, M.A. and Wilson, W.C. Replication sites and infection pathways of an arthropod-borne rhabdovirus in the biting midge, *Culicoides sonorensis.* Invited presentation, Microbial Pathogenesis: mechanisms of infectious disease, FASEB Summer Conference, Snowmass, CO, August, 2004

Mecham, J.O., White, D.M., Drolet, B.S. and Wilson, W.C. Persistence of Bluetongue Virus in the Insect Vector and its Implications for Disease Control. U.S. Animal Health Association Meeting, Greeensboro, NC, October, 2004.

Scherer, C.F.C., Golde, W.T., Gregg, D., Estes, D.M., Drolet, B.S. and Rodriguez, L.L. Vesicular stomatitis New Jersey virus in cattle: pathogenesis, virus distribution and local immune response. Conference of Research Workers in Animal Diseases, Chicago, IL, November, 2004

PUBLICATIONS:

Bonneau, K.R., Zhang, N.Z., Wilson, W.C., Zhu, J.B., Zhang, F.Q., Li, Z.H., Zhang, K.L., Xiao, L., Xiang, W.B. and MacLachlan, N.J. 2000. Phylogenetic analysis of the S7 gene does not segregate Chinese strains of bluetongue virus into a single topotype. Arch. Virol. 145:1163-1171.

Johnson, D.J., Wilson, W.C., and Paul, P.S. 2000.Validation of a reverse transcriptase multiplex PCR test for the serotype determination of U.S. isolates of bluetongue virus. Vet. Microbiol. 76:105-115.

Mecham, J.O. and Jochim, M.M. 2000. Development of an enzyme-linked immunosorbent assay for the detection of antibody to epizootic hemorrhagic disease of deer virus. J. Vet. Diagn. Invest. 12:142-145.

Wilson, W.C., Ma, H.-C., Venter, E.H., van Djik, A.A., Seal, B.S. and Mecham, J.O. 2000. Phylogenetic analysis of bluetongue viruses based on gene S7. Virus Res. 67:141-151. Erratum: Virus Res. 73:201-202. 2001.

Wilson, W.C. and Mecham, J.O. 2000. Molecular evolution of orbiviruses. Proc. U.S. Anim. Health Assoc. 104:169-180.

Meissner, J.D., Mecham, J.O. and Wilson, W.C. 2001. Verification of bluetongue virus S9 segment nu cleotide sequences. Virus Res. 81:93-101.

Duca, K.A., Lam, V. Keren, I. Endler, E.E. Letchworth, G.J. Novella, I.S. and J. Yin, 2001. Quantifying viral propagation in vitro: towards a method for characterization of complex phenotypes. Biotechnology Prog. 17:1156-1165.

Dunowska, M., Letchworth, G.J., Collins, J. and DeMartini, J.C. 2001. Ovine herpesvirus-2 glycoprotein B sequences from tissues of ruminant malignant catarrhal fever and healthy sheep are highly conserved. J. Gen. Virol. 82:2785-2790.

Gerry, A.C., Mullens, B.A., MacLachlan, N.J. and Mecham. J.O. 2001. Seasonal transmission of bluetongue virus by *Culicoides sonorensis* (Diptera: Ceratopogonidae) at a southern California dairy and evaluation of vectorial capacity as a predictor of bluetongue virus transmission. J. Med. Ent. 38:197-209.

J.O. Mecham, et al. 2001. Report of the committee on bluetongue and bovine retroviruses. Proc. U.S. Anim. Health Assoc. 105:111-121.

Mecham, J.O. et al. 2002. Report of the committee on bluetongue and bovine retroviruses. Proc. U.S. Anim. Health Assoc. 106:150-162.

Drolet, B.S., Campbell, C.L., and Mecham, J.O. 2003. Protect yourself and your sample: Processing arbovirus infected biting midges for viral detection assays and differential expression studies. In, J. Richmond (ed.), Anthology of Biosafety: Arthropod-Borne Diseases, Chapter 17.

Mecham, J.O., Stallknecht, D., and Wilson, W.C. 2003. The S7 Gene and VP7 Protein are Highly Conserved Among Temporally and Geographically Distinct American Isolates of Epizootic Hemorrhagic Disease Virus. Virus Res. 94:129-133.

Mecham, J.O., Pearson, J.E., et al. 2003. Report of the committee on bluetongue and bovine retroviruses. Proc. U.S. Anim. Health Assoc. 107:204-213.

Nunamaker, R.A., Lockwood, J.A., Stith, C.E., Campbell, C.L., Schell, S.P., Drolet, B.S., Wilson, W.C., White, D.M. and Letchworth, G.J. 2003. Grasshoppers can serve as reservoirs and vectors of vesicular stomatitis virus. J. Med. Ent. 40:957-963.

McLaughlin, B.E., DeMaula, C.D., Wilson, W.C.; Boyce, W.M. and MacLachlan, N.J. 2003. Bluetongue and epizootic hemorrhagic disease virus infections of pulmonary artery endothelial cells from cattle, sheep and deer. Am. J. Vet. Res. 64:860-865.

McHolland, L.E. and Mecham, J.O. 2003. Characterization of cell lines developed from field populations of Culicoides sonorensis (Diptera: Ceratopogonidae). J. Med. Ent. 40:348-351.

McLaughlin, B.E., DeMaula, C.D., Wilson, W.C.; Boyce, W.M., MacLachlan, N.J. 2003. Bluetongue and epizootic hemorrhagic disease virus infections of pulmonary artery endothelial cells from cattle, sheep and deer. Am. J. Vet. Res. 64:860-865.

O'Toole, D., Perez de Leon, A.A., Hearne, C., McHolland, L., Yun, L., Tabachnick, W. 2003. Papular dermatitis induced in guinea pigs by the biting midge Culicoides sonorensis (Diptera: Ceratopogonidae). J. Vet. Diagn. Invest. 15: 67-71.

Mecham, J.O., and Wilson, W.C. 2004. Antigen capture competitive enzyme-linked immunosorbent assays using baculovirus-expressed antigens for diagnosis of bluetongue virus and epizootic hemorrhagic disease virus. J. Clin. Microbiol. 2004. 42:518-523.

Wu, S. and Letchworth, G.J. 2004. High Efficiency Transformation by Electroporation of Pichia pastoris Pretreated with LiAc and DTT. BioTechniques. 36:152-154.

Wilson, W.C., Stallknecht, D.E. and Mecham, J.O. 2004. Field-deployable real-time PCR detection of bluetongue and epizootic hemorrhagic disease viral RNA. 3rd International OIE Symposium on Bluetongue. (In Press).

White, D.M. and Mecham, J.O. 2004. Lack of detectable bluetongue virus in skin of seropositive cattle: Implications for vertebrate overwintering. 3rd International Symposium on Bluetongue. (In Press).

Balasuriya, U.B.R., Nadler, S.A., Wilson, W.C., Smythe, A.B., De Santis, P., Zhang, N. and MacLachlan, N.J. 2004. Global isolates of bluetongue virus segregate into region-specific topotypes based on phylogenetic analysis of their NS3 gene. 3rd International OIE Symposium on Bluetongue Virus. (In Press).

Mecham, J.O., White, D.M., Drolet, B.S. and Wilson W.C. 2004. Persistence of Bluetongue Virus in the Insect Vector and its Implications for Disease Control. Proc. U.S. Anim. Health Assoc. (In preparation).

Arthropod-Borne Animal Diseases Research

Project Title: Development and Validation of Rapid Field Deployable Genetic Tests for Vesicular Stomatitis Virus
CRIS Number: 5410-32000-012-00X
Scientists: Wilson, W., Mayer, R., Letchworth III, G.
Location: Laramie, Wyoming
Arthropod-Borne Animal Diseases Research
Contact: Wilson, W., Phone: (307) 766-3622, Fax: (307) 766-3500
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PROJECT OBJECTIVES:

The objectives of this research project are to: 1) generate basic epidemiological information about vesicular stomatitis virus (VSV) occurrence in endemic areas in southern Mexico and Costa Rica for development and design of field validation study; 2) perform field validation of VSV rapid diagnostic tests in well-documented infected and non-infected cattle herds in Mexico and Costa Rica.

The approach will include the establishment of sentinel herds in selected areas of southern Mexico and Costa Rica with various levels of documented clinical VS activity. Basic information about previous history of clinical disease will be collected at the beginning of the study in order to determine the historical occurrence of VSV at each farm. Rapid diagnostic tests developed at Plum Island Animal Disease Center (PIADC) and Arthropod-borne Animal Diseases Research Laboratory (ABADRL) will be evaluated in laboratory. The most promising tests will then be validated in the field. Collaborative teams will carry out rapid diagnostic tests in the field during periods of high VSV activity previously identified by this study. Viruses from clinical cases as well as environmental or arthropod samples will be genetically characterized at PIADC and ABADRL.

OVERALL PROJECT ACCOMPLISHMENTS:

This project was supported by one-time funding from Homeland Security and was initiated in 2003. Clinical samples were collect from endemic area in Mexico and Central America. Over 600 of these samples were processed for VI with immuno-histochemistry verification and rapid real-time PCR. One group of samples was missed by the real-time PCR. Sequence analysis of the virus from these samples was used to modify the real-time PCR. The final validation has been delayed due to facilities failures at the ABADRL.

IMPACT:

The potential for introduction or emergence of indigenous insect transmitted diseases necessitates rapid and accurate methods to detect and measure the severity of infection by arborviruses. The ABADRL with Plum Island Animal Disease Center collaborators have developed a rapid real-time nucleic acid amplification assay (RT-PCR) VSV. Laboratory diagnostic validation of the VSV test was favourable and will differentiate VSV infection from Foot and Mouth Disease Virus, however, field validation resulted is some false negative results. The test has been modified based on these samples and the new test is currently being evaluated and was utilized to investigate the outbreak of VSV in Texas, New Mexico and Colorado the

summer of 2004.. This test will enhance certification of animals and germplasm for international trade and the ability to monitor the extent of a disease outbreak. This technology could rapidly be developed in case of an outbreak of an emerging arboviral pathogen, an intentional or un-intentional introduction or random evolutionary change in an indigenous virus.

TECHNOLOGY TRANSFER:
Provided training on real-time PCR for detection of vesicular stomatitis virus to Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica and Laboratorio de Diagnóstico de Enfermedades Vesiculares, Panama City, Panama, May 2004.

PUBLICATIONS:

Rodriguez, L.L., Pauszek, S.J., Smoliga G. and Wilson, W.C. 2004. Phylogeny-Based Multiplex Real-Time RT-PCR for Rapid Detection of Vesicular Stomatitis Viruses NJ and IN-1 from Diverse Geographical Regions. Proc. U.S. Anim. Health Assoc. (In preparation).

Letchworth, G.J., Jiménez, C., Herrero, M., Cornish, T.E., Wilson, W.C., Smoliga, G. S., Pauszek, J., Dornak, C., George, M., Rodriguez, L.L. 2004. Validation of a Real-Time RT-PCR for Vesicular Stomatitis Virus. Proc. U.S. Anim. Health Assoc. (In preparation).

Poisonous Plant Research
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Poisonous Plant Research, Logan, UT

- Investigation of Lupine-Induced Crooked Calf Disease in the Northwest
- Poisoning of Livestock by Various Larkspur Species(Delphinium)

Poisonous Plant Research Projects, Logan, UT

Project Title: Investigation of Lupine-Induced Crooked Calf Disease in the Northwest
CRIS Number: 5428-32000-011-01S
Scientists: James, L., Pfister, J., Ralphs, M., Panter, K.
Location: Logan, Utah
Poisonous Plant Research
Contact: James, L., Phone: (435) 752-2941, Fax: (435) 753-5681
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PROJECT OBJECTIVES:
Lupines continue to cause significant losses to the livestock industry in the western U.S. from induced 'crooked calf syndrome'. In the spring of 2003, large losses were reported in California, Oregon, Idaho, Western Canada and Washington State. We have done extensive research over the last 3 years in the Scabland region of eastern Washington where lupines are widespread and cause large losses every year to cattle producers. The teratogenic lupine identified was L. leucophyllus which contains relatively large concentrations of the teratogenic alkaloid anagyrine. Lupine-induced 'crooked calf disease' is responsible for significant losses to cattle producers in the western U.S. every year. In the last five years increasing incidences of the disease have been reported. In 1997, a massive loss occurred in the scablands of Washington State. Three studies have been initiated to answer significant questions. (1) what factors contribute to the increased consumption of lupine plants resulting in large losses; (2) what animal factors contribute to the increased incidence of 'crooked calf disease'; (3) which range areas are high risk for large losses. A combination of cows native to the Scabland region and naive cows have been used in the grazing studies.

OVERALL PROJECT ACCOMPLISHMENTS:
Lactating cows were fed Lupinus leucophyllus at Washington State University. Extraction and analytical protocols were developed in our laboratory for analysis of lupine alkaloids in milk. Significant levels of teratogenic alkaloids were detected in the blood and milk of cows fed L. leucophyllus and absorption and elimination profiles were determined in blood and milk.

Analyses were completed from grazing trials in Washington State with fat and thin cows. There was a significant correlation between body condition and when cattle started to graze lupines and how long they grazed lupine plants. Eventually all animals ate substantial quantities of lupine, but thin animals ate more lupine at times, thus we believe the risk of intoxication is particularly high for thin cows compared to cattle with higher body condition scores. Data analyses were completed and published from an earlier cooperative research project with the University of Idaho at the Dubois Sheep Experiment Station that demonstrated that thin sheep more rapidly absorbed toxic alkaloids than did fat sheep, and conversely thin sheep showed slower elimination of alkaloids compared to fat animals. This strongly suggests that toxicokinetics of the teratogenic alkaloids may be significantly altered based on animal body condition.

In grazing seasons 2001-2003 cows started eating lupine around the first of July after annual cheatgrass had matured and dried out, and other forbs were grazed out or matured (Lupine consumption ranged from 17 to 25% of diets). This period of consumption overlaped the critical

period of gestation (40-70 day of pregnancy) if the traditional breeding season begins May 1. Cattle should be removed from lupine areas during this period.

IMPACT:

TECHNOLOGY TRANSFER:
Information about alkaloid content, susceptible stages of gestation and relative risk of grazing in specific pastures has been transferred to extension agents and ranchers through meetings, phone conversations and written recommendations. Many management options are currently available based on available knowledge about which alkaloids in the plant are a threat, susceptible stages of gestation, effective herbicides and management techniques to reduce losses.

Management to reduce livestock losses from poisonous plants. Influence of drought on losses to toxic plants. Presented at the Arizona Strip Workshop, March 26, 2003, Kanab Utah, and March 27, 2003, St. George, Utah. Sponsored by Arizona and Utah Extension Services. The information presented at these workshop is available to ranchers and is being implemented in many grazing programs.

List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: This does not replace your peer-reviewed publications listed in Question 9).

by Michael Ralphs, Kip Panter, Jim Pfister, Clive Gay and Ernie Motteram. 2003. Cattle Consumption of lupine in the scablands of Eastern Washington, Presented at Society for Range Management annual meeting, Feb 2, Casper WY.

Panter, K.E. 2003. Presented seminar on toxic and teratogenic plants at 'Plant Interactions and the Effect of Global Warming on Alpine Biodiversity' workshop in Kazbegi, Republic of Georgia, June 30-July 6.

Pfister, J.A. 2003. Presented two workshops at the Arizona Strip, March 26, 2003, Kanab Utah, and March 27, St. George, Utah. Sponsored by Arizona and Utah Extension Services.

PUBLICATIONS:

Panter, K.E., James, L.F., Wang, S., Gardner, D.R., Waffield, W., Molyneux, R.J., Stegelmeier, B.L. and Bunch, T.D. 2003. Use of Bovine Embryos Produced by In Vitro Fertilization Techniques to Screen Poisonous Plant Toxins for Cytotoxicity, In: T. Acamovic, C.S. Stewart and T.W. Pennycott, eds. CABI Publishing, Oxon, UT.

Poisonous Plant Research, Logan, UT

Project Title: Poisoning of Livestock by Various Larkspur Species(Delphinium)
CRIS Number: 5428-32630-009-00D
Scientists: James, L., Lee, S., Gardner, D., Pfister, J.,ralphs, M., Stegelmeier, B. Panter, K., Vacant
Location: Logan, UT, Poisonous Plant Research
Contact: James, L, Phone: (435) 752-2941, Fax: (435) 753-5681, lfjpprl@cc.usu.edu

PROJECT OBJECTIVES:

Larkspur (Delphinium spp.) poisoning causes serious economic loss to livestock producers grazing cattle on many western ranges. The objectives of the multidisciplinary research are: (1) isolate and identify individual alkaloids from Delphinium species; test these alkaloids for toxicity; confirm structure activity relationships for new alkaloids; (2) develop analytical and diagnostic methods for larkspur alkaloids, including development of immunogenic alkaloid protein conjugates and antibody based detection methods and potential vaccine development; (3) investigate mechanisms of intoxication, including toxin metabolism and clearance, and treatment of intoxicated animals; and (4) determine plant, animal, or environmental factors influencing larkspur consumption and continue development of management strategies to reduce losses for all classes of larkspur.

OVERALL PROJECT ACCOMPLISHMENTS:

Developed the Fourier transform infrared spectroscopic method of analysis of toxic and total alkaloid levels in tall larkspur, thus allowing for rapid analysis of thousands of samples.

Elucidated the comparative toxicity of 24 alkaloids from larkspurs and structure activity relationships. Understanding toxicity has important implications for developing rational treatments to reduce losses.

Developed grazing strategies to reduce losses to tall larkspurs. Continued implementation of early- and late-season grazing strategies has resulted in reduced cattle losses for ranchers grazing on tall larkspur-infested pastures.

Determined that cattle eat tall larkspur in a cyclic "toxification-detoxification" pattern after ingesting a daily threshold of 14-18 mg toxic alkaloid/kg b.w. This research helps to understand patterns of consumption and how much larkspur is necessary to poison cattle.

Determined that when cattle eat larkspur, adverse postingestive consequences regulate consumption, not plant flavor (i.e., taste and smell). Increases in the toxic alkaloid concentration deter consumption by sheep, but have little impact on short-term consumption by cattle. This research provides fundamental information about when and why cattle eat larkspurs.

Determined that mineral supplementation does not affect the amount of larkspur consumed by grazing cattle, indicating that producers can save money by providing mineral supplements as necessary for nutritional needs, rather than using minerals to alter larkspur consumption.

Developed aversive conditioning as a potentially viable tool for ranchers. This practice could potentially greatly reduce the risk of cattle losses for those livestock producers that implement aversive conditioning.

Developed herbicidal control recommendations for tall larkspurs. Ranchers with dense populations of larkspur can greatly reduce losses and enhance economic returns through the use of herbicides.

Verified that sheep grazing before cattle can reduce larkspur availability and acceptability to cattle grazing afterward.

Collaborative work with Colorado State University investigated the mechanism of action of MLA, nudicauline, 12-deacetylnudicauline, barbanine, and deltaline. It was determined that the alkaloids block nicotinic acetylcholine (nAch) receptors postsynaptically. The potency of deltaline is much lower, indicating that deltaline does not bind as tightly as do the other alkaloids at nAch receptors.

IMPACT:

TECHNOLOGY TRANSFER:
Toxic larkspur alkaloid analyses done on a number of plant samples for ranchers and extension personnel. This is a useful tool for ranchers to help determine if they can begin grazing with low risk, or continue to graze, a particular pasture or allotment.

The management concepts referred to as "primary toxic window" and "early season grazing" have been developed and transmitted to numerous ranchers and USFS personnel. These concepts are based on plant chemistry and cattle diet selection patterns, and are being used to reduce losses on summer-grazed Forest Service allotments where tall larkspur is problematic.

PUBLICATIONS:

Gardner, D.R., Ralphs, M.H., Turner, D., Welsh, S. Taxonomic Implications of Diterpene Alkaloids in Three Toxic Tall Larkspur Species (Delphinium SPP.). Biochemical Systematics and Ecology. 2002.

Li, X., Gardner, D.R., Ralphs, M.H., Wang, R. Development of STS and CAPS Markers for Identification Three Tall Larkspurs (Delphinium SPP.). Genome. 2002.

Pfister, J.A., Ralphs, M.H. Gardner, D.R., Stegelmeier, B.L., Manners, G.D., Panter, K.E. and Lee, S.T. 2002. Management of three toxic Delphinium species based on alkaloid concentrations. Biochemical Systematics Ecology 30:129-138

Panter, K.E., Manners, G.D., James, L.F., Gardner, D.R., Ralphs, M.H., Pfister, J.A., Stegelmeier, B.L., Lee, S.T. Larkspur Poisoning: Toxicology and Alkaloid Structure Activity Relationships. Biochemical Systematics and Ecology. 2002.

Panter, K.E., James, L.F., Gardner, D.R., Ralphs, M.H., Pfister, J.A., Stegelmeier, B.L., Lee, S.T. Reproductive Losses to Poisonous Plants: Influence of Management Strategies. Journal of Range Management. 2002.

Pfister, J.A., Gardner, D.R., Stegelmeier, B.L., Knight, A.P., Waggoner, Jr., J.W. and Hall, J.O. 2002. Plains larkspur (Delphinium geyerii) grazing by cattle in Wyoming. Journal of Range Management 55:350-359.

Li, X., Ralphs, M.H., Gardner, D.R., Wang, R.R.-C. 2002. Genetic variation within and among 22 accessions of three tall larkspur species (Delphinium spp.) based RAPD markers. Biochemical Systematics Ecology, 30:91-102.

Welch, S.L. and M.H. Ralphs. 2002. Some tall larkspurs (Delphinium-Ranunculaceae), a taxonomic review. Biochemical Systematics Ecology 30:103-112.

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PROJECTS

Poultry Research

- Diagnosis and Control of Mycoplasmosis in Poultry

Poultry Research Projects

Project Title: Diagnosis and Control of Mycoplasmosis in Poultry
CRIS Number: 6406-32000-006-00D
Scientists: Branton, S., Vacant, Vacant, Vacant
Location: Mississippi State, MS, Poultry Research
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PROJECT OBJECTIVES:
Mycoplasmas negatively impact both interstate movement and international export of poultry and are a major factor in limiting production in both meat-type and layer-type chickens. This research seeks to define the impact of the various poultry mycoplasmas, to develop test procedures to identify infected flocks, and to develop new vaccines against the organism.

OVERALL PROJECT ACCOMPLISHMENTS:
Major accomplishments over the life of the project include: 1) the finding that Mycoplasma gallisepticum isolation occurs more frequently (300% increase) and with greater ease from the palatine fissure in the roof of the chicken¿s mouth as compared to traditional tracheal isolation, impact - fewer swabs are required to isolate the organism and swabs are easier to obtain with less stress to the chicken, 2) the finding that the chicken remains infected over its life and that neither isolation nor disinfection of its environment will rid the chicken of the organism, impact - unless you can depopulate an infected farm, you cannot get rid of the organism from the flock, 3) that the cellular profile of the chicken's blood reflects an acute infection with mycoplasma; however, the cellular blood profile of the chronically infected chicken is similar to control hens, despite the fact that the organism can still be isolated from the chronically infected hen, impact - the chicken's immune system is apparently "tricked" by the organism into believing there is no need to continue mounting an immune response, 4) that the addition of the commonly used therapeutic agent ammonium chloride in layer chickens hinders the bacteriological recovery of Mycoplasma gallisepticum from chickens, impact - a diagnostician needs to know that a flock is being treated with ammonium chloride when he is attempting to isolate the organism and therefore take a greater number of swabs in order to assure the organism's isolation when the flock is actually infected, and 5) that vaccination of chickens at 10 weeks of age with the F strain of Mycoplasma gallisepticum does not reduce egg production over a 44-week laying cycle, impact - since 1979, flock managers have used the F strain of Mycoplasma gallisepticum with the understanding that the vaccine reduces egg production by approximately 7 eggs/hen over a 45-week laying cycle.

IMPACT:
The work addresses these objectives under our OSQR Project Plan: optimization of current MG vaccination protocols for layer hens; the design and production of an efficacious recombinant MG vaccine by molecular technologies for use in egg-type (layer hen) and potentially meat-type (broiler and turkey) production systems; and to determine the mechanisms by which MG vaccinations induce immunological change. New and improved regimens for use with currently available vaccines will be the initial products of this research. Concurrently, a recombinant vaccine(s) effective against MG will be developed. The molecular technologies used to develop

the recombinant vaccine are also anticipated to provide information regarding the pathogenicity of MG.

TECHNOLOGY TRANSFER:
A self-propelled poultry vaccinating machine was developed which delivers Mycoplasma gallisepticum vaccines uniformly and consistently and was transferred to commercial table-egg producers. The technology is undergoing field trials which should end in August 2003, at which time, the technology will be delivered to the poultry industry in both refereed journal and popular press article. There are no constraints to the adoption durability of this technology.

PUBLICATIONS:

Peebles, E.D., Parker, T.A., Branton, S.L., Willeford, K.O., Jones, M.S., Gerard, P.D., Pharr, G.T., Maslin, W.R. Effects of an S6 Strain of *Mycoplasma gallisepticum* inoculation before beginning of lay on the Leukocytic characteristics of commercial layers. Avian Diseases. 2004. v. 48. p. 196-201.

Wan, X., Branton, S.L., Hanson, L.A., Pharr, G.T. Identification and initial characterization of a putative *Mycoplasma gallinarum* leucine aminopeptidase gene. Current Microbiology. 2004. v. 48. p. 32-38.

Wan, X., Branton, S.L., Hughlett, M.B., Hanson, L.A., Pharr, G.T. Expression and subcellular location of a leucine aminopeptidase of *Mycoplasma gallinarum*. International Journal of Poultry Science. 2004. v. 3(1). p. 70-74.

Burnham, M.R., Peebles, E.D., Branton, S.L., Jones, M.S., Gerard, P.D. Effects of F-Strain *Mycoplasma gallisepticum* inoculation at twelve weeks of age on the blood characteristics of commercial egg laying hens. Poultry Science. 2003. v. 82. p. 1397-1402.

Burnham, M.R., Peebles, E.D., Branton, S.L., Walzem, R.L., Gerhard, P.D. Effects of F-Strain *Mycoplasma gallisepticum* inoculation on serum very low density lipoprotein diameter and fractionation of cholesterol among lipoproteins in commercial egg-laying hens. Poultry Science. 2003. v. 82. p. 1630-1636.

Branton, S.L., Bearson, S.M.D., Bearson, B.L., Maslin, W.R., Collier, S.D., Evans, J.D., Miles, D.M., Pharr, G.T. *Mycoplasma gallinarum* infection in commercial layers and onset of fatty liver hemorrhagic syndrome. Avian Diseases. 2003. v. 47. p. 458-462.

Burnham, M.R., Peebles, E.D., Branton, S.L., Maurice, D.V., Gerard, P.D. Effects of F-strain *Mycoplasma gallisepticum* inoculation at twelve weeks of age on egg yolk composition in commercial egg laying hens. Poultry Science. 2003. v. 82. p. 577-584.

Parker, T.A., Branton, S.L., Jones, M.S., Peebles, E.D., Gerard, P.D., Willeford, K.O., Burnham, M.R., Maslin, W.R. Effects of an S6 strain of *Mycoplasma gallisepticum* challenge before beginning of lay on various egg characteristics in commercial layers. Avian Diseases. 2002. v. 46. p. 593-597.

Pharr, G.T., Branton, S.L., Hanson, L.A., Minion, F.C., Hughlett, M.B., Wan, X. Characterization of the pMGA genes from the F-Strain (vaccine strain) of *Mycoplasma gallisepticum*. International Journal of Poultry Science. 2002. v. 1(4). p. 63-73.

Peebles, E.D., Branton, S.L., Burnham, M.R., Gerard, P.D. 2003. Influences of supplemental dietary poultry fat and F-Strain *Mycoplasma gallisepticum* infection on the early performance of commercial egg laying hens. Poultry Science. 2003. v. 82. p. 596-602.

Branton, S.L., Bearson, S.M.D., Bearson, B., Lott, B.D., Maslin, W.R., Pharr, G.T., Collier, S.D., Boykin, D.L. The effects of 6/85 live Mycoplasma gallisepticum vaccine 6/85 in commercial layer hens over a 13-week laying cycle on egg production and selected egg quality parameters and egg size distribution when challenged before beginning of lay. Avian Diseases. 2002. v. 6. p. 423-428.

Burnham, M.R., Branton, S.L., Peebles, E.D., Jones, M.S., Lott, B.D., Yeatman, J.B., Whitmarsh, S.K., Gerard, P.D. Digestive and reproductive organ characteristics in commercial laying hens as affected by F-strain Mycoplasma gallisepticum. Poultry Science. 2001. v. 80. (Suppl. 1) p. 136.

Burnham, M.R., Branton, S.L., Peebles, E.D., Walzem, R.C., Whitmarch, S.K., Gerard, P.D. Effects of F-strain Mycoplasma gallisepticum on serum lipids and lipoprotein profiles in commercial laying hens. Poultry Science. 2001. v. 80. (Suppl. 1) p. 15.

Burnham, M.R., Walzem, R.L., Peebles, E.D., Branton, S.L., Whitmarsh, S.K., Gerard, P.D. Effects of fasting on serum lipids and lipoprotein profiles in commercial laying hens. Poultry Science. 2001. v. 80. (Suppl. 1) p. 19.

Jones, M.S., Peebles, E.D., Branton, S.L., Willeford, K.O., Pharr, G.T., Montgomery, R.D., Parker, T.A., Whitmarsh, S.K., Maslin, W.R. Effects of an immunodulator and phasic challenges of S6 strain Mycoplasma gallisepticum and escherichia coli on egg production and egg characteristics of commercial laying hens during peak and post-peak lay. Poultry Science. 2001. v. 80. (Suppl. 1) p. 16.

Poultry Production and Products Safety Research
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PROJECTS

Poultry Production and Products Safety Research

- Evaluation of Ascites in Poultry
- Decreasing Production Related Bone Diseases in Poultry

Poultry Production and Products Safety Research Projects

Project Title: Evaluation of Ascites in Poultry
CRIS Number: 6226-32000-004-00D
Scientists: Balog, J., Donoghue, A.
Location: Fayetteville, AR, Poultry Production and Products Safety Research
Contact: Balog, J., Phone: (479) 575-6299, Fax: (479) 575-4202, jbalog@uark.edu

PROJECT OBJECTIVES:
Ascites is an often fatal metabolic disease thought to be caused by the birds' heart and lungs not being able to support the rapid growth of modern commercial broilers. The disease is characterized as congestive heart failure, with a build up of fluid in the abdominal cavity of the bird. Ascites can be experimentally induced by rearing poultry in hypobaric (low pressure) chambers that simulate high altitude by reducing available oxygen. ARS has the only large-scale hypobaric chamber (holds up to 480 birds) in the world to study ascites. This model is being used to evaluate the etiology of ascites and methods to reduce the natural occurrence of ascites in the poultry industry. Our research has demonstrated a genetic basis for ascites. Our current research effort is to identify the gene or gene products associated with the syndrome.

OVERALL PROJECT ACCOMPLISHMENTS:
Our past work on ascites syndrome in broilers is directly related to Animal Health National Program, NP 103 by virtue of the disease aspect, and since it is generally accepted that ascites syndrome has a large genetic component our work is also related to the Genetic Improvement component of Food Animal Production National Program, NP 101. While the influence of genetics in the etiology of the disease has been recognized for years, lines of broilers that can serve as experimental models were not available. Our most significant accomplishment over the life of this project is that we, along with our collaborator Dr. Nicholas B. Anthony of the University of Arkansas, have developed two lines of birds that vary in their ascites response to high altitude (low oxygen pressure) and have collected tissue samples from both lines of birds. Genetic selection has resulted in divergent line responses, with the ascites-resistant line exhibiting 26% ascites mortality, while the ascites-susceptible line exhibits 98.5% ascites mortality. These lines of birds and their tissue samples will be used to determine if genetic markers exist that could be used to improve breeder selection for ascites resistance.

Over the past several years, we have fine-tuned the hypobaric chamber experimental model for inducing ascites. Previous hypobaric chambers were only able to accommodate 15 birds at a time. Our chamber can hold 480 birds to six weeks of age, thereby greatly increasing the sensitivity of our experiments. Additionally, results of recent studies show that birds need not be maintained at high altitude for the entire 6 weeks grow-out to produce a high incidence of ascites mortality and that bird numbers can be maximized and equipment failure can

be compensated for by moving birds out of the chamber. Once the experimental model was established, we investigated several ways to reduce ascites mortality in broilers. We have found that dietary aspirin at fairly low levels can reduce ascites. Since dietary aspirin would be an inexpensive and relatively easy method to use in commercial poultry operations, there is great potential to impact broiler production. We have determined that correct timing of feed restriction can reduce ascites mortality, without sacrificing final body weight. This finding was particularly important because feed restriction is commonly used in the poultry industry, yet frequently the timing is incorrect, which results in birds being delayed going to market.

Our recent investigations of the molecular basis of ascites syndrome have resulted in several major accomplishments. We were awarded a grant from the U.S. Poultry and Egg Association (#58-6226-2-0002) for our proposal titled 'Molecular Genetics of Ascites Syndrome (Pulmonary Hypertension Syndrome) in Broilers' to conduct research to determine if mutations in the coding region of the bone morphogenetic protein receptor 2 (BMPR2) were linked to ascites susceptibility in broilers. Utilizing commercial broiler crosses and the ascites-susceptible and ascites-resistant lines, we sequenced the BMPR2 messenger RNA (mRNAs) of ascitic and non-ascitic birds and then compared these sequences with the published BMPR2 gene. No mutations were found in the mRNA that altered the BMPR-II protein, but 13 single nucleotide polymorphisms (SNPs) were detected, and we determined that BMPR-II protein levels are increased in ascitic birds. Although it appears that ascites is not caused by the same mutation that causes FPPH, regulation of BMPR2 expression is different in birds that develop ascites and we now have 13 SNPs that can be useful in gene mapping.

IMPACT:
Utilizing the world's largest capacity hypobaric chamber for poultry, we, along with our collaborator Dr. Nicholas B. Anthony of the University of Arkansas, have developed two lines of birds that vary in their ascites response to high altitude (low oxygen pressure) and have collected tissue samples from both lines of birds. Genetic selection has resulted in divergent line responses with the ascites-resistant line exhibiting 26% ascites mortality, while the ascites-susceptible line exhibits 98.5% ascites mortality. These lines of birds and their tissue samples are being used to evaluate the molecular etiology of ascites and to determine if genetic markers exist that could be used to improve breeder selection for ascites resistance. Continued molecular work into the etiology of ascites syndrome led us to report that there are significant differences in the levels of seven electron transport proteins in ascitic birds compared with non-ascitic birds. We also identified 14 single nucleotide polymorphisms in the coding region of chicken bone morphogenetic protein receptor II and mapped this gene to the chicken chromosome 7. We also determined that mutations in the chicken bone morphogenetic protein receptor II gene of the chicken are not responsible for ascites susceptibility.

TECHNOLOGY TRANSFERS:
The molecular genetic component of ascites syndrome is of great interest to the poultry industry, particularly the primary poultry breeding companies, because of this a Cooperative Research and Development Agreement (CRADA) was established with Cobb-Vantress, Inc. This CRADA 'Characterization of the Genetic Basis for Ascites Syndrome' supports an in-depth investigation of the molecular aspects of ascites susceptibility by utilizing the ascites susceptible and resistant lines of broilers. This CRADA allows for greater interaction between ARS scientists and Cobb-

Vantress, Inc., a primary poultry breeding company, and has potential to benefit the poultry industry as a whole.

PUBLICATIONS:

Balog, J. M., Anthony, N. B., Cooper, M. A., Kidd, B. D., Huff, G. R., Huff, W. E. and Rath, N. C. Ascites syndrome and related pathologies in feed restricted broilers raised in a hypobaric chamber. Poultry Sci.79:318-323. 2000.

Balog, J.M., Huff, G. R., Rath, N. C., and Huff, W. E. Effect of dietary aspirin on ascites in broilers raised in a hypobaric chamber. Poultry Sci. 79:1101-1105. 2000.

Balog, J.M., Anthony, N. B., Kidd, B. D., Liu, X., Cooper, M. A., Huff, G. R., Huff, W. E., Wideman, R. F., and Rath, N. C. Genetic selection of broiler lines that differ in their ascites susceptibility. 2. Response of the ascites lines to cold stress and bronchus occlusion. 13th World Poultry Science Association European Symposium on Poultry Nutrition. Blankenberge, Belgium. pp. 329-330. 2001. (Proceedings)

Anthony, N.B., Balog, J. M., Hughes, J. D., Stamps, L., Cooper, M. A., Kidd, B. D., Liu, X., Huff, G. R., Huff, W. E., and Rath, N. C. Genetic selection of broiler lines that differ in their ascites susceptibility. 1. Selection under hypobaric conditions. 13th World Poultry Science Association European Symposium on Poultry Nutrition. Blankenberge, Belgium. pp. 327-328. 2001. (Proceedings)

Cisar, C. R., Balog, J. M., Anthony, N. B., and Donoghue, A. M. Genetic selection of broiler lines that differ in their ascites susceptibility 3. Progress on identification of differentially expressed genes in ascitic and non-ascitic birds. 13th World Poultry Science Association European Symposium on Poultry Nutrition, Blankenberge, Belgium. pp. 331-332. 2001. (Proceedings)

Balog, J. M., Kidd, B. D., Huff, W. E., Huff, G. R., Rath, N. C., and Anthony, N. B. Effect of cold stress on broilers selected for resistance or susceptibility to ascites syndrome. Poultry Sci. 81:1383-87. 2003.

Balog, J. M. Ascites syndrome (pulmonary hypertension) in broiler chickens: Are we seeing the light at the end of the tunnel? Avian and Poultry Biology Reviews, 14(3):99–126. 2003.

Cisar, C. R., Balog, J. M., Anthony, N. B., and Donoghue, A. M. Sequence analysis of bone morphogenetic protein receptor type II mRNA from ascitic and non-ascitic commercial broilers. Poultry Sci. 82:1494-99. 2003.

Cisar, C. R., Balog, J. M., and Donoghue, A. M. The chicken bone morphogenetic protein receptor type II (BMPR2) gene maps to chromosome 7. Animal Genetics 34:475-476. 2003.

Project Title: Decreasing Production Related Bone Diseases in Poultry
CRIS Number: 6226-32000-002-00D
Scientists: Rath, N., Donoghue, A.
Location: Fayetteville, AR, Poultry Production and Products Safety Research
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PROJECT OBJECTIVES:
Leg problems are major metabolic skeletal problems in meat-type poultry often affecting fast-growing chickens and turkeys. Tibial dyschondroplasia (TD) is a common growth plate problem in which the proper development cartilage is impaired, preventing bone development at the growing end of leg bones. Consequently the growth plate and bone become fragile, prone to deformities and infections. The etiology of TD is unknown but it is a complex disease since it can be related to a chondrocyte development, vascularization, cartilage modeling, cell survival, and differentiation that are regulated by a number of nutritional, physiological, and environmental factors. We are trying to understand the cellular and biochemical basis of TD so that it can be controlled by appropriate dietary interventions. We are developing experimental models of TD that can be used to understand the mechanisms of the disease and screen the dietary supplements that can prevent the problem in growing poultry.

OVERALL PROJECT ACCOMPLISHMENTS:
Over the past several years we have studied poultry leg problem and their causes particularly relating to tibial dyschondroplasia (TD). We have also done some work on the aspects of poultry immunity, particularly on the role of certain blood proteins called 'acute phase protein' that modify immunity and affect disease resistance. We have demonstrated that TD is caused by an unusual cell death in the growth plate, which affects cartilage replacement by bone in healthy animals. In investigating the causes of poultry skeletal problems, we also described a phenomenon of subchondral infarct in turkeys that may lead to an initial infection of bone leading to turkey osteomyelitis. Because bacteria tend to colonize dead tissues, dead cartilage in TD-affected bones may facilitate infection, resulting in diseases, such as osteomyelitis. Our research also showed that a commonly used pestiicide (thiram) known to increase the incidence of TD does so by causing chondrocyte death. To understand the physiological sequences of TD, we have currently developed a model with which we can induce TD in susceptible birds with only two days of feeding with thiram, which we hope will lead to a better understanding of the mechanisms of TD and design methods for its prevention. Prevention of unusual cell death in the growth plate which appears to be a key factor in the mechanisms TD, may lead to the reduction of the incidence of TD in the future. We also showed that bone strength is better related to the content of bone collagen cross-links than to bone minerals, and that younger birds have substantially lower content of collagen cross-links. This gives us the opportunity to improve bone strength by manipulating collagen cross-link content of bone. We also developed a novel staining procedure for avian heterophils, which facilitates their fluorescent localization in tissues. A bioassay of avian interleukin-6 was developed in our lab. We identified ovotransferrin as an avian acute phase protein. We developed an ELISA for its measurements in blood and showed its immunomodulating properties. This research provides a tool for the diagnosis of health problems and stress in poultry. It also provides opportunity to investigate the role of ovotransferrin as an immunomodulating agent. We identified the presence of matrix metalloproteinase (MMP) in bile as an exocrine product and hypothesized its possible function in

digestive physiology. Currently, we have developed a new chicken macrophage cell line that can be used to better understand the innate immune process in poultry.

IMPACT:
Comparing age dependent changes in different bone constituents to bone strength we found that pyridinium crosslinks of collagen are better related to bone strength raising the possibility that improving collagen crosslinks will improve bone strength. We showed that tibial dyschondroplasia (TD), a metabolic cartilage defect is caused by a premature cell death in the maturing zone cartilage which is a basis of this problem. We have developed an experimental model of TD using a pesticide thiram, which induces the disease in young chickens following a brief exposure to this agent. This model will enable to explore the cellular and molecular mechanisms of TD and can be used to determine if certain nutrients can protect against the disease. Leg problems cost poultry industry an economic loss in excess of $200 million dollars annually in USA. By understanding the mechanism and the etiology of these problems it will be possible not only prevent economic losses but also improve production efficiency and welfare. We developed a bioassay method for avian interleukin-6, a novel fluorescent staining method for avian heterophils, and a new chicken macrophage cell line HTC. We identified serum ovotransferrin as a major avian acute phase protein that has immunomodulatory effects and can be used as a diagnostic indicator of poultry health problems. These studies provide basic tools to understand the basis of poultry health and disease resistance that will improve health and well being.

TECHNOLOGY TRANSFERS:
Our research is made available to the public through publications in journals, news magazines, presentations in meetings and conferences, and personal communications with other scientists and poultry growers. We have provided the macrophage cell line HTC to researchers in universities and government labs. We have trained students (both graduates and undergraduates) from the Poultry Science, Animal Science, and Food Science Departments at the University of Arkansas. We have provided our knowledge of bone disease, the analytical methods to academicians and representatives of poultry industries.

PUBLICATIONS:

Rath, N. C., Bayyari, G. R., Balog, J. M. Huff, W. E. Physiological studies of turkey tibial dyschondroplasia, Poult. Sci., 1994 73: 416-424.

Rath, N. C., Bayyari, G. R., Beasley, J. N., Huff, W. E. and Balog, J. M. Age related changes in the incidence of tibial dyschondroplasia in turkeys. Poult. Sci., 73: 1254-1259, 1994.

Rath, N. C., Huff, W. E., Bayyari, G. R. and Balog, J. M. Identification of transforming growth factor-β and interleukin-6 in chicken ascites fluid. Avian Dis. 39: 382-389, 1995.

Rath, N. C., Huff, W. E., Balog, J. M., Bayyari, G. R. and Reddy, P. R. Matrix metalloproteinase activity in avian tibial dyschondroplasia. Poult. Sci. 76: 501-505, 1997.

Rath, N. C., Huff, W. E., Balog, J. M. and Bayyari, G. R. Cell death in avian tibial

dyschondroplasia. Avian Dis. 42: 72-79, 1998.

Rath, N. C., Huff, G. R., Balog, J. M. and Huff, W. E. Fluorescein isothiocyanate staining and characterization of avian heterophils, Vet. Immunol. Immunopathol. 64: 83-95, 1998.

Rath, N. C., Huff, G. R., W. E., Huff, and Balog, J. M., Factors regulating bone maturity and strength in poultry. Poultry Sci. 79:1024-1032, 2000.

Xie, H., Huff, G. R., Huff, W. E, Balog, J. M., Holt, P., and Rath, N. C. Identification of ovotransferrin as an acute phase protein in chickens. Poultry Sci. 81:112-120. 2002.

Xie H, Newberry L., Clark F.D., Huff, W. E., Huff, G. R., Balog, J. M., Rath, N. C. Changes in serum ovotransferrin levels in chickens with experimentally induced inflammation and diseases. Avian Dis. 46:122-131. 2002.

Xie, H., Huff, G. R., Huff, W. E., Balog, J. M., Rath, N.C. Effects of ovotransferrin on chicken macrophages and heterophil-granulocytes. Dev. Comp. Immunol. 26:805-815. 2002.

Rath, N. C., 2003. Tibial dyschondroplasia, a poultry leg problem. Feed Info. www.feedinfo.com/asp/scientific/science_res.asp?id_sci=2120 10/7/2003.

Rath, N.C.,Parcells, M., Xie, H. and Santin, E. Characterization of a spontaneously transformed chicken mononuclear cell line, Vet. Immunol. Immunopathol. 96:93-104. 2003.

Rath, N.C., Huff, W. E., Balog, J. M., and Huff, G. R. Comparative efficacy of different dithiocarbamates to induce tibial dyschondroplasia in poultry. Poultry Sci. 83: 266-274. 2004.

Rath, N. C., Huff, W. E., Huff, G. R., and Balog, J. M. Mechanisms of thiram-induced tibial dyschondroplasia in chickens. World's Poultry Congress, 2004.

Rath, N.C., Balog, J.M., Huff, W.E., Huff, G.R. Chondrocyte maturation in thiram-induced tibial dyschondroplasia in poultry. Proceedings of XIII Congress of the World Veterinary and Poultry Association. 2003. p. 114.

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