West Nile Virus

VETERINARY MEDICAL EXPERIMENT STATION
COLLEGE OF VETERINARY MEDICINE
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25th Annual Report

Enhancing animal production, profitability, and well-being by improving animal health.

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The Veterinary Medical Experiment Station (VMES) supports a wide range of research that impacts on almost all aspects of our lives, from the food we eat and the clothes we wear, to our physical, emotional, and economic health, to the quality of our environment. VMES research includes efforts to improve the productivity and health of poultry and livestock, to better the quality of life for companion animals, and to improve public health through disease surveillance. This year’s research is profiled in our 2000-2001 VMES annual report.

VMES funds are intended to help develop extramurally funded research programs at the College of Veterinary Medicine. In addition, VMES funds are used to support short-term applied research that directly benefits the health of animals and livestock in Georgia. Projects supported by VMES funds are evaluated for scientific merit, importance to animal health, consideration for experimental animal welfare, and their roles in meeting the research objectives of the VMES.

Our objectives are as follows:

- To improve the health and productivity of domestic livestock, poultry, fish, and other income-producing animals and wildlife through research;
- To assist in preventing disease epidemics by providing laboratory resources and highly skilled scientific personnel;
- To assist in protecting human health through the control of animal diseases transmissible to man;
- To improve the health of companion animals, which serve to enrich the lives of humankind;
- To train new scientists in animal health research in order to provide continuity and growth in this area of veterinary medicine.
# Table of Contents

VMES Objectives ............................................. 2

Report of the Director ........................................ 4

West Nile Virus .................................................. 5

Poultry .............................................................. 6

Fish ................................................................. 12

Ruminants ......................................................... 13

Equine ............................................................. 15

Companion Animals ........................................... 16

Comparative Biomedicine ..................................... 18

Financial Highlights .......................................... 20
  Research Funding .............................................
    Georgia Livestock and Poultry: Inventories and Values
    Georgia Farm Cash Receipts

Research Contracts and Grants ............................. 21

Administrators and Advisors ............................... 23

Researchers ...................................................... 24

Selected Publications ........................................ 26

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All programs and activities of the Veterinary Medical Experiment Station are conducted without regard to race, color, national origin, age, sex, or handicap.
This is the 25th Annual Report of the Veterinary Medical Experiment Station, and I am pleased to report that the Experiment Station continues to achieve its mission of applying basic and applied research for the improvement of animal and public health. Although our mission remains constant, I am sure everyone who reads this realizes that the field of veterinary research has changed dramatically over the last 25 years. Veterinarians and animal scientists have been compelled to rethink many issues during this period. For instance, the rise of antibiotic-resistant strains of bacteria has changed the way antimicrobial agents are used to treat and prevent infectious diseases in animals. Increasing public concern over the use of experimental animals has heightened scientists’ awareness to more effectively communicate how research directly benefits the health of animals as well as humans. New technologies, such as therapeutic and diagnostic monoclonal antibodies, genetic vaccines, and rapid polymerase chain reaction (PCR) diagnostic tests which were unheard of 25 years ago, are now readily available for protecting animal health. The rapid acquisition and annotation of the entire DNA sequence from genomes of animals and microbial pathogens is revolutionizing veterinary and human medicine. And finally, whereas some diseases such as brucellosis have been eradicated from Georgia, others such as Johne’s disease have newly emerged or are reemerging to plague animal and human populations.

We can be sure that profound changes resulting from the rapid development of biotechnology and the occurrence of new diseases will continue over the next 25 years. Now as much as ever our mission remains critical to the people of Georgia. The VMES plays a primary role in supporting research on animal health problems of present and future concern to our state’s livestock and poultry industries as well as its wildlife resources. Our food animal industries are valued now at well over $3 billion. Sales of livestock, poultry, and their products account for more than half of Georgia’s annual farm income. It behooves us to protect these resources. A continued commitment at the state level to support research on animal health is a smart investment, particularly in view of the fact that there is limited federal and private funding targeted specifically for animal health research.

Station researchers, using a science-based approach, addressed many challenging animal health problems this year in areas ranging from the emergence of antimicrobial resistance in veterinary pathogens to the mechanisms of fish immunity. The 25th Annual Report provides an overview of VMES-supported projects and research in the College of Veterinary Medicine during the fiscal year of 2000-2001.

The cover of this year’s Annual Report by Chief Medical Illustrator Kip Carter and the accompanying article by Drs. Daniel Mead, and David Stallknecht highlight West Nile virus. As you will read, mosquitoes transmit West Nile virus (WNV) from viremic birds to uninfected birds and other animals, including horses and humans. This pathogen was found in Georgia for the first time in 2001, and the number of cases is expected to increase. Wildlife biologists and veterinarians in the Southeastern Cooperative Wildlife Disease Study (SCWDS) and the VMES at the College of Veterinary Medicine conduct research on the epidemiology of this emerging disease. This important work will benefit both human and animal populations in our State.
Much has been written in the popular press about West Nile Virus in the past two years and the virus has been reported in Georgia for the first time this year. West Nile virus (WNV) is closely related to St. Louis encephalitis virus that has long been present in the United States. However, WNV was recognized in the Western Hemisphere for the first time in the late summer of 1999. At that time, a wildlife pathologist and a zoo veterinarian in the New York City area noticed an unusual number of deaths in wild crows and birds in a zoo collection. Their investigations, including postmortem examinations of dead birds, yielded the first recovered WNV in the United States. This information was used to identify WNV as the cause of a simultaneous outbreak of human cases of viral encephalitis.

WNV is transmitted by mosquitoes from infected birds to uninfected birds, humans, horses, and other animals. As with other arboviruses [arthropod-borne viruses (arthropods include insects, ticks, and mites)], WNV activity generally disappears along with the insect vectors when autumn brings colder weather. Most humans exposed to WNV via mosquito bites do not become ill at all. However, a small percentage of infected persons have mild flu-like symptoms, from which they readily recover. Clinical neurologic disease is found in an even smaller percentage of infections, and few cases, primarily in the elderly or persons with a decreased immune system, may result in fatal inflammation and swelling of the brain. Vaccination for humans against WNV infection currently is unavailable, and avoidance of mosquito bites is the primary mode of prevention.

The distribution of WNV has been increasing since it was first detected in the New York City area. The outbreak of 1999 eventually involved Connecticut, Maryland, and New Jersey before it ceased with the onset of cold weather. Approximately 60 patients were hospitalized with WNV infections in the New York City area, and seven persons died. Many wild birds died and 25 cases were diagnosed in horses. In 2000, WNV was documented in more than 4,000 wild birds in the states with WNV in 1999, as well as in New Hampshire, Vermont, Rhode Island, Pennsylvania, Washington, DC, Virginia, and North Carolina. Two of 21 human cases proved fatal. In addition to wild birds, WNV infections were diagnosed in 57 horses and a handful of rabbits, squirrels, raccoons, bats, cats, and a chipmunk in the New York City area.

Georgia’s first confirmed case of WNV was found in a dead crow from Lowndes County examined at the College of Veterinary Medicine’s Southeastern Cooperative Wildlife Disease Study (SCWDS) on July 10, 2001. The crow was examined as part of the wild bird surveillance that SCWDS conducts under an agreement with the Georgia Department of Human Resources’ Division of Public Health. The Department of Pathology and the Department of Medical Microbiology and Parasitology also are participating in this surveillance effort. The Athens and Tifton Diagnostic Laboratories are conducting surveillance of domestic animals, primarily horses. More than 3,500 birds have been tested for WNV at SCWDS since the program began in July 2000, and the number continues to rise. As of late August 2001, WNV has been detected in 81 dead birds from 25 counties around Georgia, including additional southern counties where WNV has been confirmed in two horses and the metro Atlanta area where the only fatal human infection in the United States has occurred this year. The number of affected Georgia counties continues to increase.

WNV activity also has been detected this year in wild birds from several states affected in previous years, plus Florida, Alabama, and Louisiana in the South and Ohio, Indiana, and Michigan to the north, as well as in Windsor, Ontario. Florida also has confirmed three nonfatal human cases as well as WNV infections in 12 horses. However, dead wild birds remain the best indicators of WNV activity in an area. Infection has been documented in more than 55 native avian species in the United States since 1999. Crows and jays are highly susceptible, and surveillance is concentrated on these species. Wildlife biologists and veterinarians working with public health officials continue to play a key role in detecting the presence of WNV. Ongoing surveillance throughout the country undoubtedly will document expansion of the range of this emerging pathogen in the United States.

—Dr. David Stallknecht
—Dr. Daniel Mead
Georgia’s poultry industry dominated the state’s animal agricultural dollars with nearly $2.7 billion in annual revenue in 1999. The state’s poultry industry is continuing to expand as broiler production in Georgia increased. The urbanization of northern Georgia is causing the broiler expansion to occur primarily in the state’s southern section. Because of the intensive management system, poultry producers are emphasizing disease prevention. VMES scientists have responded to industry demands by developing vaccines to prevent infectious diseases. Scientists are also helping to improve poultry health by developing inexpensive, rapid, and accurate methods for disease diagnosis. Although the primary poultry health concerns are respiratory diseases, recent efforts have been initiated to control type J avian leukemia virus, a major cause of the tumor, myeloblastosis. Researchers are also focusing on the reduction of potential human pathogens on poultry products nationwide and on ways to prevent the development of resistance against antibiotics.

Investigation into Factors Affecting Hatchability and Chick Quality

The effectiveness of hatchery disinfectants under use conditions may not correlate with laboratory data. For this reason, disinfectant efficacy was evaluated under conditions similar to those present in commercial poultry hatcheries. The marker Escherichia coli was inoculated onto the surface of fertile hatching eggs, which were then incubated according to industry practices. A commercial quaternary ammonium compound (QAC), mixed at the manufacturer’s recommended dilution rate, was misted into one incubator during the incubation period. A second machine received a mist of the same QAC at the same concentration but mixed with EDTA-tris at a 1:3 ratio. The third incubator received deionized distilled water in the same volume as the disinfectant. Air samples were taken during the incubation period to assess the concentration of aerosolized bacteria within the machines. Upon hatching, all unhatched eggs were broken open and evaluated for the cause of their failure to hatch. A selection of one-day-old chicks was weighed, killed, and yolk sacs weighed and cultured. Chicks were reared according to industry practices in floor pens and were evaluated at one week of age for body weight, yolk sac weight, and bacterial contamination and at two weeks of age for mortality and feed conversion.

The incubator receiving unpotentiated QAC had the highest bacterial load, but the potentiated disinfectant group had a bacterial load similar to the nondisinfectated machine. Contamination of the unhatched eggs and day-old chicks followed this same pattern. The eggs exposed to the potentiated QAC had a higher level of late embryonic deaths including pipped eggs. Day-of-age and one-week-of-age body weights and yolk sac weights were not affected by treatment nor were the two-week livability or feed conversion rate.

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Bactericidal Efficacy of Avian β-defensins Against Food-borne Pathogens in Poultry

Natural antimicrobial peptides such as β-defensins are important components of innate disease resistance in animals. We found that β-defensins are produced in avian white blood cells and in some mucosal tissues. Expression of β-defensins was detected by Northern blot analysis in lungs, conjunctiva, and bursas from broiler chickens. Increasing expression of β-defensins may be a way to improve disease resistance in broilers and to reduce the carriage of food-borne pathogens in poultry. Recently, we synthesized the DNA that codes for a chicken β-defensin. Next, we will insert the DNA into a mammalian expression vector such that we can evaluate the bactericidal efficacy of this peptide against food-borne pathogens and against avian pathogens. Once the antimicrobial spectrum of the β-defensins is known, the feasibility of improving β-defensin expression in poultry can be evaluated as a means of enhancing disease resistance and reducing carriage of food-borne pathogens in poultry.

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Control of Infectious Bronchitis Virus (IBV)

Infectious bronchitis is a highly contagious upper-respiratory tract disease of commercial poultry that is difficult to control and causes severe economic losses to the poultry industry. Based on genetic analysis of a recombinant IBV isolate (DE072), we found that it had high nucleotide similarity in some genes, but other individual genes had a much different level of sequence similarity. These data were used to identify regions that may serve as recombination hot spots, which are important for epidemiological studies of this virus. In another study, we used genetic, serologic, and in vivo analysis, and identified a group of IBV isolates as a new serotype Georgia 98 (GA98). With that knowledge, we developed a diagnostic test that can differentiate this new serotype from all other serotypes of IBV such that
it can be easily identified. The spike glycoprotein of IBV is translated as a precursor protein (So) then cleaved into two subunits (S1 and S2) by host-cell serine proteases. We compared the cleavage site of 55 IBV isolates and determined that the cleavage site sequence, which consists of five basic amino acid residues, does not correlate with host cell range, serotype, or pathogenicity. However, it does correlate with geographic origin. In addition, the number of basic residues around the cleavage site does not appear to correlate with increased cleavability, host cell range, and increased virulence as it does with envelope glycoproteins in orthomyxoviruses and paramyxoviruses. We have successfully expressed the entire spike gene in LMH cells. Furthermore, we have demonstrated that a virus-neutralizing monoclonal antibody against a conformationally dependent epitope recognized the expressed protein, indicating that faithful reproduction of the virus neutralizing epitope was obtained. This result is important for the development of a spike subunit vaccine against IBV.

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Rapid Identification and Epidemiological Typing of Food-borne Pathogens by Polymerase Chain Reaction (PCR)

We have developed a strategy for detection of food-borne pathogens in foods involving an initial screen using multiplex polymerase chain reaction enzyme-linked immunosorbent assay (PCR-ELISA). A multiplex PCR was designed using Campylobacter jejuni fliB, Escherichia coli O157 rfbB, and Salmonella invA genes as target sequences for PCR. The OD values from PCR-ELISA for several C. jejuni, E. coli O157 isolates and Salmonella serotypes, including Salmonella enteritidis and Salmonella typhimurium, were at least 2 standard deviations greater than the negative controls, commensal or other non-O157 E. coli isolates. Repeating multiplex PCR and identifying the size of the PCR amplicon determined the identity of the food-borne pathogen. We have also developed a molecular serotyping scheme for Salmonella that can be performed by any laboratory with a PCR thermocycler. A multiplex PCR was designed for detection of five major Salmonella O serogroups (A/D1, B, C1, C2, and E1). Primers were created to amplify gene sequences unique to specific serogroup, producing a PCR amplicon unique in size for that Salmonella serogroup. Signal of the expected size was generated only from multiplex PCR of Salmonella belonging to five major O serogroups. The multiplex PCR was used to identify which enrichments were positive for Salmonella. Sixteen of twenty Salmonella culture-positive samples were positive by PCR. Results of the PCR also corresponded with the identity of O the serogroup determined for Salmonella isolated from the enrichment. Combining PCR-ELISA with this multiplex PCR brings us closer to real-time identification of food-borne pathogens in a contaminated product.

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Avian Mycoplasmosis

The avian mycoplasmas, Mycoplasma gallisepticum (MG), Mycoplasma synoviae (MS), Mycoplasma meleagridis (MM), and Mycoplasma iowae (MI) are egg-transmitted infections causing respiratory, reproductive, and joint and tendon disease in chickens and turkeys. The objectives of this study were to improve detection and control measures for avian Mycoplasma infection, to study their pathogenesis, and to determine the incidence of avian mycoplasmas by DNA fingerprinting.

We are continuing studies of methods to develop more effective tools for molecular epidemiology. Previous results have shown that random amplification of polymorphic DNA (RAPD) is useful, but results may be variable and difficult to interpret. Use of a second or even a third primer set may be required to differentiate among closely related strains. We have since concentrated on the pvpA, mgc1, and LP genes of M. gallisepticum. Primer sets for each of these genes have been developed, and DNA sequence alignments from each has been found to be useful for differentiation of MG strains. Results generally agree with those from RAPD, but sequencing of these genes appears to be a more accurate measure of relatedness.

In the case of M. synoviae, RAPD has proven to be inconsistent. We have found that DNA sequence analysis of the vlhA gene is useful for molecular epidemiology.

Challenge studies in recent isolates of MS from turkeys have shown that such strains are virulent and incompatible with economic turkey production.

These results improve our ability to detect and control MG and other mycoplasmas in commercial poultry.

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Epidemiological Studies on Infectious Bursal Disease—Virus Field Isolates in the Southeastern United States

Despite widespread vaccination, infectious bursal disease virus (IBDV) continues to cause economic losses to the poultry industry. Within serotype 1, there are classic, variant and very virulent viruses. The U.S. poultry industry is affected most by the presence of antigenic variants that

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can be responsible for vaccination failures. The VP2 gene of IBDV has been the target for molecular classification of the virus because it is the major host protective antigen responsible for inducing serotype-neutralizing antibodies. Advancements in nucleic acid technology have led to the identification and classification of antigenic variants by reverse transcriptase-polymerase chain reaction/restriction fragment length polymorphism (RT-PCR/RFLP) analysis. In addition, potential antigenic regions have been identified within the hypervariable region of the IBDV VP2 gene that may be important for pathogenesis. Molecular analyses in this region of VP2 can be used to group these field variants based on their unique genetic properties. Recent studies have indicated a potential for segment reassortment between serotype 1 and serotype 2 in very virulent European isolates of IBDV. The objectives of this proposal are to conduct an epidemiological study of IBDV field isolates from the southeastern United States. Field isolates will be chosen for the study based on unique restriction fragment length polymorphism (RFLP) patterns obtained using the current IBDV typing system at the Poultry Diagnostic and Research Center (PDRC). We will amplify both segments (A and B) of the IBDV genome from the field viruses using reverse transcriptase-polymerase chain reaction (RT-PCR) and clone the resulting products for sequencing. Finally, phylogenetic analyses of the complete genomic sequences of the IBD variant viruses will be compared with previously published IBDV gene sequences.

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The Impact of Competitive Exclusion on Reducing the Level of Antibiotic-Resistant Escherichia coli in Poultry and their Environment

Physicians and veterinarians are facing a situation in which bacterial pathogens are becoming resistant to antibiotics faster than new drugs are developed. There is a concern that the use of antibiotics in food animals could result in antibiotic resistance in bacteria in humans. We demonstrated in the first year of this work that in isolation units an undefined bacterial culture of the ceca of chickens called competitive exclusion, or CE, given to day-old chicks could reduce the level of antibiotic resistant E. coli in the intestines of these chicks. This should result in much less environmental contamination of the antibiotic resistant E. coli.

The next phase of this work was to look at these same type of CE products in chickens raised on the floor thus more closely approximating broiler-industry conditions. However, we could no longer obtain any of these undefined CE cultures. Both Bayer (Aviguard) and Continental Grain (MSC) are now seeking FDA approval for salmonella control and cannot provide any of these cultures for research. We are therefore using a probiotic (Lactobacillus sp.) and a prebiotic (fructo-oligosaccharide) in these studies. The results of the first study do not indicate these limited bacteria CE or probiotic products will be as effective in reducing colonization and shedding of the antibiotic resistant E. coli. A second study was completed in May 2001 to determine if this negative trend is correct. If these probiotic/prebiotic products are not successful, then this research will be terminated until the undefined CE products are again available. Theundefined CE cultures may aid in reducing the level of antibiotic resistant E. coli in a broiler chicken’s environment. This may reduce the potential of resistant bacteria on chicken meat, therefore reducing the potential risk of transfer of antibiotic resistance from animals to humans.

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Development and Characterization of Infectious Laryngotraecheitis (ILT) Recombinant Virus

The main goal of this proposal is to investigate the role of glycoprotein E (gE) in the pathogenicity and tissue tropism of ILTV. To address this question, efforts to construct a gE null mutant are in progress, and plasmid constructs to engineer the recombinant virus have been developed. Second, to identify naturally existing ILTV gE mutants sequence analysis was performed for 15 strains. Analysis of predicted amino acid for two plasmid clones of each strain showed two types of mutations when compared with the gE consensus. Fix mutations were identified using the criteria that at least two of the clones from the same strain had an amino acid change at a particular position when compared with the consensus sequence. Mix mutations were defined as an amino acid change observed in one of the two clones from the same strain. Three fix and 37 mix mutations were observed among the 15 isolates analyzed. Within the 37 recorded mix mutations observed, among vaccine stains and field isolates, 3 may be significant in the gE function. These mutations were at position 153, 412, and 419. Mutation at position 153 produced a terminal codon indicating...
that some viral subpopulations may be expressing a truncated gE protein. Mix mutations at position 412 and 419 were located near the tyrosine phosphorylation motif of the gE cytoplasmic tail. These motifs are important to sort proteins within the cell and to promote cell-to-cell spread of alphaherpesviruses. Ongoing efforts to isolate these natural occurring mutants are progressing in our laboratory.

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Adaptive Responses of Avian Tendon to Injury

A biped model using Arbor Ross broilers was developed to investigate the response of the gastrocnemius tendon to loading. The study protocol consisted of two treatments: control and treadmill paced. From age 3 weeks to 6 weeks, the paced birds were exposed to a 30-minute daily treadmill walking regimen. Gastrocnemius tendons were collected at 3, 4, 5, and 6 weeks of age. Biomechanical assessment of tendon behavior was conducted by quasi-static failure testing. A cubic model was applied to test data to explain biomechanical performance of the avian gastrocnemius tendon. Significance of age and treatment was accepted at the 95 pct. level. In the control group, a significant effect of age was found at all points of investigation for tensile load at failure for the gastrocnemius tendon. Similarly, in the paced group, significant age-related effects were found for all combinations except at 5 and 6 weeks of age. The pacing treatment did not have a significant effect on tensile load at failure for the gastrocnemius tendon. Linear-regression analysis indicated a high correlation between tensile load and shank length and tibia length. R-squared was greater than 0.97 for all comparisons. Further investigation is warranted to determine the possible structural and material properties; however, the pacing treatment did not generate a significant change in toughness values. Neither age nor pacing was found to have an effect on the structural or material tangent modulus of the gastrocnemius tendons analyzed in the study. Despite the lack of statistical significance, the moduli of the control tendons were consistently higher than the structural and material moduli calculated for the paced tendons. This finding indicates that the control tendons were stiffer and theoretically stronger than their paced counterparts. In summary, modest treadmill pacing exposure has been shown in this study to produce a more compliant tendon. This would suggest that further investigations in collagen turnover, proteoglycan synthesis, and collagen fibril morphology need to be undertaken to explain the biomechanical response of avian gastrocnemius tendon to moderate exercise.

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Advancements in the Isolation, Characterization, and Control of Avian Viruses

Specific investigations involving pathogenic viruses that present a continual hazard to the commercial poultry industry are the basis of our current research. Variant strains of infectious bronchitis virus (IBV) are currently being evaluated in our laboratory. In vitro recombination events occurring between a D-072 IBV variant and commercial vaccine strains have been characterized by molecular assays. In vivo studies that have evaluated the protection afforded by commercial vaccines against a characterized California IBV variant have also been performed. Separately, adaptation studies of IBV to different tissues in order to decrease upper respiratory reactions have been successful and will be completed to prelude in vivo challenge and tissue tropism studies.

Several infectious bronchitis disease virus (IBDV) variants have been characterized via molecular assays and in vivo challenge studies. Identical studies have been concluded for chicken anemia virus isolates. In vivo coinfection characterization will be performed for these two viruses using in situ hybridization.

Two avian adenovirus strains have been propagated in the chorioallantoic sac of embryos and characterized via molecular assays. Viral stocks have been prepared for further purification and application for prepared antigen use in an enzyme linked immunoadsorbent assay (ELISA) format.

Several avian leukosis virus-subgroup-J (ALV-J) viruses have been obtained and isolated in a continuous cell line from different genetic lines, companies, and generations of chickens. A variety of these isolates have been sequenced and have produced substantial genomic information. In addition, statistical comparison of ALV-J detection sensitivity for both the established virological assay and nested reverse transcriptase-polymerase chain reaction (RT-PCR) have been performed and completed.

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Poultry

Dr. Pedro Villegas inoculates avian virus into chicken eggs. The virus will propagate in the eggs, providing Dr. Villegas and his lab with the concentrations of virus particles required for characterization studies.
The Effects of Avian Leukosis Virus-Subgroup J (ALV-J) Infections in Broilers

Our study on the effects of ALV-J infections in broilers consists of four major objectives. The first one is to determine the rates of lay, hatch, and horizontal spread of the infections. There were no significant effects on hen-day egg production, but hen-housed production was decreased 5 to 8 pct. because of increased mortality (30 pct. vs. 14 pct.). Hatch rate was depressed 10 pct. to 30 pct. Individual hen ALV-J status was stable over time with no spread from hen to hen. Progeny had lateral transmission with 100 pct. infection rate by 6 weeks of age.

Our second objective was to investigate broiler performance parameters. Positive broilers weighed less than 65 pct. of negative chicks from 1 to 8 weeks of age.

Third, we analyzed the effects of ALV-J on bone marrow, lymphocytes, heterophils, and monocytes. Bone marrow showed necrosis, hypoplasia, lymphoid nodule formation, and myeloid hyperplasia and neoplasia. Lymphocytes had no differences in mitogen responses, but there were decreased circulating T lymphocytes and altered CD4+ : CD8+ ratios. Heterophils had no change in resting numbers, had incorporated provirus, had decreased phagocytic/bacteriocidal activity, and had suppressed reactivity to Staphylococcus aureus inoculation in vivo. These heterophil results may explain the increased incidence of secondary infections seen in birds with ALV-J infection. Macrophages contained provirus, but their numbers and in vivo functions were not altered. To examine the effects on macrophages in vivo, studies examining reactivity to killed commercial vaccines are in progress.

Fourth, we produced polyclonal and monoclonal antibodies. SPF chickens injected with cloned ALV-J developed neutralizing polyclonal antibody J that was not detectable with the commercial ALV-J antibody enzyme-linked immunoadsorbent assay (ELISA) system. This means using the ELISA antibody test as an eradication tool will lead to false negative results with some ALV-J isolates. Antibodies were used to detect ALV-J in tissue sections by immunoperoxidase staining. Studies on the ability of developed neutralizing antibodies (injected in ovo) to increase resistance of chicks are in progress.

Results showed ALV-J spreads easily between broilers after hatch and only mildly suppresses heterophil and macrophage reactions to infections. Studies in progress will show if maternal/injected antibody will protect against exposure in the hatchery and broiler house and increase chick resistance to exposure.

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Investigation of Natural Disease Outbreaks

This project is an ongoing proposal that provides diagnostic laboratory support for the poultry industry, source material for research, and teaching experiences for students in the Master of Avian Medicine (MAM) program.

Serological assessment in a broiler operation with severe condemnations at processing showed significant titers against Connecticut-like infectious bronchitis virus. Addition of a Connecticut strain vaccine to the broiler vaccination program ended the condemnations and the financial losses caused by this virus.

Field investigations by professional staff and students typically lead to significant changes in disease and farm management practices, which bring solutions to difficult problems.

Improvements in the lab database continue with functional and additional data search capabilities and simplified maintenance. Lab reports are being sent by email in pdf format and faxed directly from within the system without the need for an intermediate hard copy. Offering lab data by secure web site is being investigated.

The polymerase chain reaction (PCR) technique is an integral part of the diagnostic laboratory as seen by the consistent demand for these tests. PCR techniques for infectious bronchitis virus, mycoplasma, infectious bursal disease, infectious laryngotracheitis virus, and avian leukosis virus-J (ALV-J) provide mostly same-day results. Techniques for large volume processing and faster turnaround are being evaluated.

Diagnostic Services Laboratory activity is represented by 6,361 accessions, 31,340 bacterial procedures, 210 antimicrobial susceptibilities, 49,229 enzyme-linked immunoadsorbent assays (ELISAs), 37,726 Infectious bronchitis virus-hemagglutination inhibition (IBV-HI) tests, 17,500 mycoplasma plate agglutination tests, 385 agar gel precipitin tests, 30,100 histopathology slides, 1,900 diagnostic PCR tests, and 2,093 necropsies.

Correlation of Phenotypic and Genotypic Characteristics and Embryo Lethality in Identifying Virulent and Commensal Avian Escherichia coli

The aim of our study was to determine which phenotypic and/or genotypic laboratory tests correlate with the embryo lethality assay for establishing the virulence status of avian E. coli isolates. Ten field isolates of E. coli from cases of colibacillosis and ten E. coli isolates cultured from the intestinal tracts of normal broiler chickens were assayed for selected phenotypic and genotypic characteristics. These tests included the embryo lethality assay, bacterial resistance to chicken complement, colicin activity, motility, type F1 fimbriae, and the presence of the increased serum survival (iss) and the arsH genes. The presence of Colicin-V and the increased serum survival gene (iss) are reliable tests that may be used to identify virulent and commensal avian E. coli.

Richard E. Wooley, Penelope S. Gibbs, Lisa K. Nolan, Catherine W. Giddings, Shelley M. Horne, and Steven L. Foley (wooleyr@vet.uga.edu)
Georgia’s aquaculture industry is steadily expanding, with its greatest increase occurring in channel catfish production. Pond acreage for catfish farming has continued to grow every year. Other species being developed for aquaculture include striped and largemouth bass, yellow perch, and tilapia. In addition to Georgia’s developing food-fish industry, there is an increasing interest in ornamental fish production, particularly koi, and cultured shellfish. It is estimated that aquaculture production in all countries will have to expand at least twofold to meet world demand for fisheries products over the next 25 years. Continued commercial aquaculture success will depend on increased efficiency in resource use, innovative farming methods, and a quality end product. Fish health is an essential issue at every level of fish production. As Georgia’s aquaculture industries continue to grow, research aimed at improving the health of aquatic animal species will help growers reduce production costs and improve profits.

Identification of a Fas Receptor-like Molecule in *Tetrahymena* spp.

Obligate and facultative protozoan parasite infections cause extensive losses in aquaria as well as in farm-raised marine and freshwater species of fish. Although both cellular and antibody-mediated responses occur following infection, the mechanisms of resistance to parasitic diseases remain very contentious. We have previously reported that innate immunity in the form of nonspecific cytotoxic cells (NCC) plays a direct role in the lysis of protozoan parasites. We recently demonstrated that the Fas ligand–Fas receptor (FasL–FasR) pathway of apoptosis participates in the mechanism by which NCC kill protozoan parasites during teleost innate immune responses. Before adaptive immunity can be detected, teleost NCC participate by releasing soluble Fas ligand that induces the death of the parasite. The presence of NCC and sFasL in tissues may have the most immediate and effective function in teleost regulation of anti-protozoan immunity by amplification of inflammatory responses and by induction of apoptosis in parasites expressing membrane FasR. We have gathered evidence that suggests that *Tetrahymena* spp. express a protein that is cross-reactive with anti-human FasR antibodies. The FasR-like protein binds to human recombinant sFasL, and induces apoptosis in *Tetrahymena* as shown by DNA laddering and hypoploidy. Fas-receptor antibodies recognize a protein on the membrane of the parasites as shown by flow cytometry and microscopic analysis of immunofluorescence stained cells of different strains of parasites. This is the first report of the presence of apoptosis receptors on protozoan parasites, and it could explain the mechanism of innate immune resistance to parasitic infections in fish and perhaps even in mammals.

Liliana Jaso-Friedmann
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Molecular Characterization of a *Piscirickettsia*-like Pathogen of Tilapia

In the early 1990s, tilapia in farms on the island of Oahu in Hawaii started showing signs of a new disease syndrome. This syndrome was similar to piscirickettsiosis, caused by *Piscirickettsia salmonis*, an obligate intracellular bacterial pathogen of salmonids. Tilapia infected with the *Piscirickettsia*-like organism (PLO) often swim erratically, appear to have trouble staying at depth in the water column, have occasional cutaneous hemorrhages in the skin, and often have exophthalmia (popeye). But often the first sign of disease is death. The gills show epithelial hyperplasia, with some severe consolidation of secondary lamellae. Multiple white granulomas are observed in the gills and tissues. The PLO syndrome does not form ring-shaped lesions in the liver as seen with *P. salmonis*, and the agent is active at higher temperatures. When spleens from infected tilapia were tested with a *P. salmonis*-specific FA, no fluorescence was observed. This result suggests that the PLO has different surface antigens than *P. salmonis*. The organisms are inconsistently visualized with Giemsa and Warthenstarry stains and very inconsistently with Brown and Bend Gram stain. But they stained well with Lilly stain. In blood smears, moderate to large numbers of intracellular bacteria were noted with rare circulating monocytes. Some predilection for nervous tissue including peripheral nerves, spinal cord, and brain is observed in histological sections. In TEM pleomorphic bacterial organisms, generally cocccoid in shape, approximately 0.4 – 0.67 x 0.5 – 0.89 mm were observed. The organisms had a double cell wall with no defined nucleus and variable electron dense and electron lucent areas. The organisms appear free in the cytoplasm and within phagolysosomes. Attempts are continuing to isolate the organism in cell culture and to isolate and characterize the 16S rDNA gene for phylogenetic analysis.

Michael J. Mauel and Kendall Frazier
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Cattle, sheep, and ruminants are three of Georgia’s important food-animal ruminants. They are considered ruminants because their four-chambered stomach enables them to digest copious roughage, which is inedible for direct human consumption. These three industries have gone through recent dynamic changes. The beef and dairy industries have liquidated their herds because of a relatively large cattle supply and low milk and beef prices. Today’s cattle producers are working with narrow profit margins and must watch their expenses more closely than ever. Consequently, biomedical researchers are providing these industries with ways to maintain healthy animals, which will help reduce production costs. Mastitis, Johne’s disease, pasteurellosis, pneumonia, infectious bovine rhinotrachitis (IBR), bovine virus diarrhea (BVD), parainfluenza-3 (PI-3), and leptospirosis continue to challenge the immune systems of Georgia’s cattle herds. Ruminant herd health as it pertains to food safety is also a major concern to consumers and producers. Scientists need to investigate pathogenic Escherichia coli, Salmonella, Campylobacter, and other food-borne organisms as to their origin, transmission, and prevalence.

**Cytokine and T-cell Responses to Mucosal Bovine Respiratory Syncytial Virus (BRSV) Vaccination**

In Georgia and across the United States, respiratory disease is a leading cause of sickness and death in cattle. BRSV is a common cause of this problem, particularly in young calves. Although BRSV vaccines exist, their efficacy is debatable; moreover, vaccination has at times enhanced disease. VMES-funded research conducted by Dr. Amelia Woolums and Dr. Corrie Brown has shown that calves may be better protected against BRSV by intranasal (IN) vaccination. IN vaccines have long been available to prevent disease caused by two other important bovine pathogens: infectious bovine rhinotrachitis (IBR) virus and parainfluenza type 3 (PI3) virus. Many veterinarians find IN IBR/PI3 vaccines the safest and most effective way to immunize young calves. However, no IN BRSV vaccines exist.

Research by Dr. Woolums and Dr. Brown has shown that an experimental IN vaccine protected calves against disease caused by BRSV; moreover, IN vaccination did not enhance disease. To determine what aspects of the immune response were most important for protection, Dr. Woolums and Dr. Brown measured cytokine production in vaccinated calves. Cytokines are proteins produced by immune cells to modulate the immune response. Interferon gamma (IFN-g) is a particularly important antiviral cytokine. Immune cells from calves receiving IN vaccination produced more IFN-g than cells from nonvaccinated calves. Interestingly, this was true in some regions of the immune system but not in others. Protection from BRSV was associated with specific activation of immune cells in specific areas of the body. Future research will be aimed at identifying ways to maximize the response of immune cells in regions important to BRSV protection, possibly by adding vaccine components that will directly target cells in these critical regions.

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**Molecular Characterization and Phylogenetic Analysis of Cryptosporidium spp.**

This study used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to provide molecular and phylogenetic characterization of various species and isolates of Cryptosporidium. Specimens from more than 60 cases, representing eight different host species [including bovine, caprine, ovine, equine, avian (macaw), avian (quail), prairie dog, and snake] were collected. Additionally, the bovine species were grouped according to the eight different soil types found throughout Georgia. Three genomic fragments (18s rRNA, acetyl coenzyme A synthetase, and HSP-70) were isolated. Bands retrieved are currently being sequenced. After completion of sequencing, a phylogenetic tree will be prepared, and the sequences from new species/isolates of Cryptosporidium will be accessioned in Genbank (after all bands are received). Restriction sites on
The genomes will be identified such that restriction enzymes can be used on future samples for identification and speciation of the organism.

The impact of this project is threefold. First, it will provide a statewide sampling of the isolates of Cryptosporidium that affect cattle populations in Georgia, suggesting the isolates of concern for future study. Second, it will expand our knowledge of the species variation of Cryptosporidium that exists, allowing for a better understanding of the significance of this organism to host species (including humans), especially when obtained from point water sources. Third, it will improve extraction protocols and allow for the elucidation of genomic data of Cryptosporidium from banked specimens.

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Survey of Nematodal Resistance Patterns in Northern Georgia Goat Herds

Goat farming is a rapidly growing agricultural industry in Georgia, but production losses from internal parasites threatens its current and future vitality. This problem is not unique to Georgians; parasite resistance to multiple classes of deworming compounds is one of the largest emerging health problems in sheep and goats around the world.

The intent of this study is to collect information that will define regional parasite resistance patterns such that informed recommendations can be made to help goat producers in Georgia develop intelligent, sustainable parasite control programs. To accomplish this goal, we are performing a fecal egg count reduction test (FECRT) and an in vitro larval development assay (LDA) on 10 goat farms in northern Georgia. Furthermore, we are collecting a detailed five-year history from each farmer concerning their parasite-control methods.

Because this study must be conducted during the times of the year when parasite transmission is optimal (summer, early fall, and spring), the study was suspended over the winter months. We recently resumed our research and have four more farms to test. However, we have completed analysis on five goat farms. The most striking (and worrisome) preliminary finding in this study is that ivermectin and benzimidazole resistance appears to be extremely prevalent among goat parasites in northern Georgia. These deworming compounds have been the mainstay of therapy for the past decade. Levamisole appears to be effective in most farms, but this drug has a small margin of safety. Moxidectin, a recently introduced milbemycin compound labeled for topical use in cattle, appears to be highly effective. However, this product is not labeled for goats, and it is not formulated for oral use. Furthermore, moxidectin is closely related to ivermectin in its mechanism of action; thus, side resistance is likely to develop if this compound is used extensively.

The results of the survey support our suspicion that many goat owners are not well informed concerning parasite control in goats. Knowledge concerning drug-resistance problems was scant, and many farmers are using inadequate doses of dewormers in their goats. In addition, many farmers are using the same compound year after year despite their own observations that the goats do not appear to be thriving. Pasture rotation was not routinely being used as a nonchemical parasite-control strategy. These findings indicate that a highly visible education program is needed to help producers make informed management choices that effectively control endoparasitism in their goats.

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In the past few decades, horses have reemerged as very important animal species in Georgia. In ages past, horses were concentrated on farms in rural parts of the state and were used primarily as work animals. Today horses assume many roles, ranging from companions to pleasure animals to show animals. They are used for pleasure riding, jumping, dressage, showing, cutting, and barrel racing. The goes on. Because of the increasing financial and emotional impact of the horse industry on the state, VM researchers are focusing on the mechanisms responsible for some of the most important diseases that affect horses. To dissect these mechanisms, these researchers are using state-of-the-art techniques to get at the very heart of the problems. This year’s VMES-funded study deals with the identification and characterization of genes in equine intestinal parasites that control the parasite’s ability to resist the effects of anthelmintic compounds (dewormers).

Avermectin/Milbemycin Resistance in Equine Cyathostomes: Characterization of the GluCl Gene Family

Control of gastrointestinal parasites remains one of the most important health-related concerns facing the equine industry. This problem has recently been magnified by the increased prevalence of anthelmintic resistance, which is recognized globally as one of the greatest health threats to grazing livestock. Extensive reliance on chemical control for equine cyathostomes (small strongyles) has led to the development of resistance to all classes of available anthelmintics except the avermectin/milbemycins (ivermectin, moxidectin; AM). However, most parasitologists agree that it is a question of when, not if, resistance to this drug class will appear. When resistance to the AM drugs develops in the cyathostomes, the frequent and widespread movement of horses will lead to rapid dispersion of resistant parasites, causing the incidence of morbidity and mortality from parasitic disease to rise dramatically.

The glutamate-gated chloride (GluCl) channel in nematodes is the putative receptor molecule and target of the AM drugs. Several recent studies have demonstrated that selection at this gene likely plays an important role in the development of resistance. Furthermore, studies in Caenorhabditis elegans have recently demonstrated that mutations in GluCl genes are sufficient to confer ivermectin resistance. Gaining basic knowledge on the biology of the equine cyathostome GluCl homologs is, therefore, a critical first step in understanding potential molecular mechanisms of AM resistance.

Over the past year, we have cloned and sequenced two separate GluCl genes from Cylicocyclus nassatus (a cyathostome parasite that commonly infects horses). We have also prepared a C. nassatus cDNA library that we are continuing to screen for further GluCl homologs. To our knowledge, this is the first cDNA library that has ever been prepared for a cyathostome parasite. Characterization of these genes is expected to provide the framework necessary to establish the genotypic basis for resistance to AM drugs that will enable the future development of molecular assays to detect the emergence of resistant worms. This is expected to have significant positive effects on equine health, because it will enable the detection of anthelmintic-resistant cyathostomes on individual horse farms or in individual horses, providing a means to prevent and control their spread.

Ray M. Kaplan
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Companion animals reside in 55 million US homes. These animals include an estimated 66 million cats, 58 million dogs, 88 million fish, 40 million birds, 13 million small animals (rabbits, hamsters, and gerbils), and 8 million reptiles. The increasing recognition of the close bond between people and their pets has magnified the importance of insuring the quality of our pets’ lives. Because of medical advances, companion animals are living longer than their predecessors. Longer life, however, means more age-related diseases and ailments, such as cancer, neural degeneration, kidney dysfunction, poor circulation, and decreased respiratory and cardiac capacity.

However, unlike other research areas, there are no federal funds and only limited state funds to support projects specifically for companion animals. The VMES has been useful in assisting new clinical faculty in their initial research projects, but the vast majority of funding has come from foundations and private industry. Industrial money has been awarded based upon the potential knowledge gained from studying companion animals with diseases comparable to diseases found in humans. Examples of externally funded projects include urinary incontinence, diabetes mellitus, renal disease, pain relief for arthritis, transdermal fentanyl patches for pain relief, feline bartonellosis and herpesvirus, feline AIDS, and minimally invasive surgery.

Development of Canine in vitro Fertilization and Embryo Transfer

Assisted reproductive technology is the treatment of choice for subfertility and infertility problems in humans. The application of these techniques has ensured that couples with little or no prospects of having a child can add their unique blend of genetic diversity to the human gene pool. Assisted reproductive technologies include in vitro maturation and fertilization of ova, intracytoplasmic sperm injection (ICSI) of ova, and embryo transfer. Collectively, these include several additional in vitro techniques including sperm collection and capacitation and in vitro culture of embryos to the multicellular stages. Considerable progress in the application of these technologies to other species has resulted in the actual cloning of an animal. However, progress has been exceedingly slow in the dog. One major reason for this may be that the dog, unlike other species, ovulates an immature ova that remains in the oviduct for up to 48 hours before maturation. Additionally, little is known about the natural hormonal environment of the oviduct and the tissue interactions that may play a role in the natural maturation process.

The primary objective of this study is to define reproducible in vitro maturation conditions for canine ova and possibly produce the first puppy using either in vitro fertilization or intracytoplasmic sperm injection and embryo transfer. Specific aims include manipulation of the hormonal environment of the ova to mimic the hormonal environment of estrus. Capacitation conditions for sperm, in vitro fertilization, and ICSI techniques will be refined to produce fertilized ova. It is anticipated that these technologies will be applicable as research tools to examine genetic problems as well as a clinical service to alleviate subfertile or infertile problems among our clientele at the teaching hospital and our referral cases.

Hugh Dookwah and Richard Fayrer-Hosken (hdookwah@vet.uga.edu)

A Canine Model of Picalic Acid-induced Dilated Cardiomyopathy-Phase II

Dilated cardiomyopathy (DCM) is one of the most common acquired heart diseases in dogs, and the most common primary heart disease in people, accounting for 60 percent of all cardiomyopathies. It is also the most common discharge diagnosis in U.S. hospitals for patients older than 60 and remains the most common indication for cardiac transplantation, at an estimated cost of $177 million annually. Long-term prognosis remains poor for most dogs and people diagnosed with DCM. Development of new treatments is often hindered by the lack of optimal animal models that closely mimic the natural form of this disease.

The cause of DCM is unknown in most dogs and people; however, one known cause in humans, dogs, and hamsters is carnitine deficiency. Carnitine, an amino acid derivative, is essential for generating energy in the heart from fat. Many causes for carnitine deficiency have been reported, including administration of drugs that contain pivalic acid (PA), a branched-chained fatty acid used to enhance intestinal absorption of drugs. In a two-month pilot study by the investigators, we were able to reliably reduce plasma
carnitine concentrations in healthy Beagles by administering oral PA, and we observed cardiac changes suggestive of very early DCM. The purpose of this study was to determine whether administering PA for a longer period of time to healthy Beagles could reduce cardiac and skeletal muscle carnitine concentrations as well as result in more definitive cardiac changes.

Six healthy adult Beagles were selected for study. Dogs received oral PA daily. Preliminary results show that three of six dogs developed reduced cardiac contractility. Paired t-test showed a statistically significant (p<0.05) reduction in fractional shortening percent and ejection fraction. Plasma and tissue carnitine concentrations are not now available to determine whether the changes observed in the heart correlate with reduced cardiac tissue concentrations.

Based on preliminary results, daily oral PA administration does result in statistically significant changes in cardiac function compatible with early DCM. When all results are available, if reduced cardiac function correlates with reduced carnitine concentrations in the heart, PA would appear to primarily affect the heart, and therefore, would have the potential of inducing DCM if administered on a longer term basis.

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Pharmacokinetics and Tissue Distribution of Nucleoside Analogs in Cats

Feline immunodeficiency virus (FIV) is similar to human immunodeficiency virus type 1 (HIV-1) in replication, genomic organization, and clinical disease manifestations. FIV infection may be more common in domestic cats in the United States than previously thought; the American Veterinary Medical Association task force on FIV and feline leukemia virus (FeLV) recently recommended routine testing for all cats, regardless of age. Our studies have focused on evaluating the potential effectiveness, in the central nervous system, of drugs that inhibit the enzyme responsible for FIV replication, called reverse transcriptase. We have finished studies testing 2 reverse transcriptase inhibitors, or nucleoside analogs, called AZT and 3TC. These drugs are commonly used in the treatment of humans with HIV-1 infection; thus, results of our studies may have important implications for the treatment of both domestic cats and humans.

Studies have been completed that measured the level of drug in the bloodstream after oral, intragastric, and intravenous administration and in the cerebrospinal fluid (CSF) after intravenous administration. AZT penetrated into the CSF better than did 3TC. Analysis of data generated from additional studies to assess the penetration of drugs into tissue in various regions of the brain is currently being completed. These data will be evaluated further using a newly generated mathematical model that we designed based on the results of an experiment to measure blood volume in various regions of the brain of normal cats. Our final results will indicate how well AZT and 3TC move out of the bloodstream within the brain and into the brain tissue where the virus hides. The information effected by these studies in normal cats will be the foundation of future studies on the effectiveness of treatment of FIV-induced central nervous system disease.

M. A. Stevenson and F. Douglas Boudinot (masteven@vet.uga.edu)

Development of a New Generation of Rabies Vaccines for Dogs and Cats

Prevention of animal rabies by vaccination not only protects animals from diseases but also reduces the public health threat to humans. Conventional rabies vaccines for dogs and cats are made from inactivated rabies virus. Although these vaccines are safe and efficacious, multiple immunizations are required to maintain adequate immunity. Because adjuvant is used, these inactivated vaccines may induce unwanted side effects ranging from local skin lesions to feline sarcoma. Therefore, a new generation of more potent rabies virus vaccines (for example, avirulent rabies virus vaccines) is needed. By using the newly developed reverse genetics technology, we constructed and selected the recombinant rabies viruses with mutations on the phosphorylation site of the nucleoprotein. Preliminary studies indicate that mutation of the nucleoprotein at the phosphorylation site leads to inhibition of virus replication. Propagation of these viruses indicate that these mutant viruses grow poorly in infected cells, and the virus yield for one of these viruses (L16SA) is at least 4 log units (10,000 times) lower than the wild-type virus. These data indicate that the growth of the mutant virus L16SA is drastically restricted, indicating attenuation of this virus. Such attenuated virus could be developed as an avirulent rabies virus vaccine for animals if inoculation of this virus into animals does not induce disease but can still induce protective immune responses. An avirulent rabies virus vaccine will induce a long-lasting immunity in cats and dogs and therefore reduce the frequency of immunizations. It will provide better protection for animals and consequently reduce health risks for humans. It will also save money for pet owners.

Zhen F. Fu (zhenfu@vet.uga.edu)
Comparative biomedicine investigates how a particular disease affects one species versus another, that is, how a disease manifests itself in say a mouse versus a human. Researchers can compare diseases between species because different species often share substantial genetic information. Scientists study data such as symptoms, disease progression, treatments, mortality, and so on. Thus, one species serves as a disease model for another. And interestingly, both species may benefit. For example, researchers study cardiomyopathy in dogs and humans and both have benefited in the short- and long-term. In the abstracts that follow, VMES scientists discuss their research using comparative biomedicine involving hypertension, malaria, toxicology, and respiratory disease.

### Immunity to Malaria During Pregnancy in Inbred Mice

There is a well-established link between pregnancy and exacerbation of or increased susceptibility to infectious diseases. Malaria is one infection for which increased susceptibility in both human and rodents has been extensively reported. Although immunomodulation of pregnancy may be responsible for this increased susceptibility, the specific immunologic changes in pregnant animals that compromise anti-parasite immunity have not been well characterized.

Because mouse models are readily accessible and manipulable, mice have been widely used to characterize the pathogenesis of malaria. Few studies, however, have attempted to perform detailed characterizations of immune responses to malaria during pregnancy in these animals or to identify the physiologic changes that modulate immune responses to malaria during gestation. The main aims of this project are the following: to identify an inbred mouse-murine malarial parasite combination that will allow us to characterize cytokine production and lymphocyte dynamics in malaria-infected pregnant mice. And second, correlate these immune parameters with disease outcomes, including course and density of parasitemia and birth outcome (i.e., litter size and pup weight) as well as physiological parameters such as hormone levels.

Our efforts in this project have revealed that *Plasmodium chabaudi* AS infection in C57BL/6 inbred mice is an appropriate model for these studies. Whereas *Plasmodium berghei* XAT, *P. berghei* NK65, *Plasmodium yoelii* 2CN and *P. chabaudi adami* infections in these same mice are not suitable because of lethality or other problems with the source parasite stocks. Furthermore, preliminary flow cytometric work has revealed that there are specific changes in T lymphocyte activation profiles (as determined by CD44 cell surface expression), on day 10 of infection/day 10.5 of pregnancy as compared with uninfected pregnant and infected nonpregnant female mice. Peripheral blood samples from pregnant infected mice also contained a higher proportion of CD8+ cytotoxic T cells at 10 days p.i. than infected nonpregnant mice, although there was no observable difference in the level of parasitemia between these groups during the first 10 days post-infection. This

### S-nitroso cysteine Recognition Sites in Hypertension

The overall hypotheses driving this proposal is that (1) the S-nitrosothiol, L-S-nitrosocysteine (L-SNC), is an endothelium-derived relaxing factor (EDRF) in resistance arteries controlling arterial blood pressure; and (2) L-SNC activates stereoselective recognition sites that may represent a super-family of membrane-bound receptors.

The principal aim of this proposal is to establish that diminished endothelium-dependent vasodilation in spontaneously hypertensive (SH) rats, an excellent model of human essential hypertension, is caused by the down-regulation of these recognition sites in vascular smooth muscle.

The principal findings include the following. (1) The vasodilator potency of L-SNC was markedly reduced in small mesenteric and femoral arteries from SH rats compared with arteries from normotensive, age-matched Wistar Kyoto (WKY) rats. (2) Vasodilator potencies of nitric oxide (NO) and the membrane-permeable cGMP-analogue, 8-CPT-cGMP, were similar in SH and WKY arteries. (3) The disulfide bond-reducing agent, dithiothreitol, markedly improved the potency of L-SNC in SH but not WKY arteries. (4) Dithiothreitol did not affect the potencies of NO or 8-CPT-cGMP. (5) Endothelium-dependent dilataion elicited by agonists (e.g., bradykinin acetylcholine) or mild hypoxia (12 pct. O2) was markedly improved by dithiothreitol.

These findings strongly support our contention that the L-SNC is an EDRF and that the vasorelaxant actions of this S-nitrosothiol are diminished in SH arteries because of the oxidation of critical cysteine residues in stereoselective S-nitrosothiol recognition sites in vascular smooth muscle of resistance arteries. This work has immediate implications with respect to the development of potential novel therapeutic strategies to treat hypertension.

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preliminary work provides promising evidence that the C57BL/6-P. chabaudi AS model system will lead to important gains in our understanding of immunity to malaria during pregnancy.

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Role of Intracellular Oxidants in Adhesion Molecule Expression on Airway Epithelium

Respiratory disease in poultry, cattle, and swine continue to take a financial toll on Georgia and the Southeast’s food-producing industries. In addition, farm workers experience repeated exposure to farm dusts that may lead to respiratory diseases, causing additional expense to the industry via medical cost and lost work time. Understanding mechanisms involved in the development of inflammatory respiratory diseases could lead to therapeutics and preventatives of importance to Georgia food-producing industries. Numerous inflammatory mediators are found in the respiratory tract in pulmonary diseases characterized by airway inflammation, such as chronic bronchitis, bronchiectasis, or even bacterial and viral infections. Substances like tumor necrosis factor alpha (TNFa) and reactive oxygen species (ROS), produced mainly by infiltrating inflammatory cells, can have several local deleterious effects in the airway. We have demonstrated that airway epithelial cells express intercellular adhesion molecule-1 (ICAM-1) and produce other cytokines that can recruit additional inflammatory cells to the airways.

ICAM-1 contributes to inflammation in the airways by increasing adherence of neutrophils to the epithelium via binding to inflammatory cell integrins. Our laboratory has demonstrated that TNFa induces ICAM-1 gene and surface expression on airway epithelial cells. Additionally, our preliminary data demonstrate that this increased expression was inhibited by the addition of antioxidants. Thus, it appears logical to look at intracellular antioxidant enzymes as potential mediators of ICAM-1 expression. This proposal will investigate the involvement of intracellular antioxidant enzymes in pathways associated with TNFa induced ICAM-1 expression in the epithelium. Investigations into the signaling pathways involved in adhesion molecule regulation are essential to understanding their role in lung diseases. These studies will further define the regulating factors controlling TNFa enhanced expression of adhesion molecules in the lung, which will benefit animal health by providing potential therapies for inflammation associated airway disease.

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The Use of Solid Phase Microextraction in Sample Preparation for GC/MS Analysis

The purpose of this project is to modify existing instrumentation and to develop procedures using solid phase microextraction (SPME) for the detection of chemicals of importance to veterinary toxicology. Sample preparation by conventional liquid-liquid extraction methods is often time intensive, labor intensive, and often involves the use of toxic organic solvents. SPME is a solventless extraction procedure that involves the exposure of a small, coated fused silica fiber to a liquid sample or to the headspace above a liquid or solid sample. Exposing the fiber to the increased temperature and gas flow of the gas chromatography injection port causes the analyte to desorb from the fiber and enter the analytical column directly. Because of delays in notification, funding for this project was made available in January 2001.

The first objective of the proposal was to modify the analytical instrument and programming software to accommodate the proposed SPME procedures. Toward this objective, the author completed an advanced training course for the gas chromatograph/mass spectrometer (GC/MS) in January 2001. Modifications of the instrument followed but were delayed because of company back orders. Final installation of instrument modifications was accomplished on May 10, 2001. Thus, very little time was available for the remaining objectives before this report was drafted.

The second objective of the proposal was to develop SPME protocols for the detection of zinc phosphide and metaldehyde in clinical cases of animal poisoning. Zinc phosphide is a rodenticide that has been involved in animal poisonings but is quite difficult to detect by standard methodologies. Metaldehyde is a snail and slug bait that has been involved in animal poisonings. Upon ingestion, both zinc phosphide and metaldehyde break down under the acid conditions of the stomach, forming volatile compounds that are difficult to detect. Although only a few procedures have been performed to date, the use of several different SPME fibers for the detection of chemical standards of zinc phosphide and metaldehyde breakdown products has been accomplished. Further work is needed to optimize the analytical conditions (injection and column time and temperature, time of extraction, optimal SPME fiber) before spiked samples of gastric contents (used to simulate diagnostic specimens) are begun. Procedures will be documented by the generation of a standard operating procedure. Because of the delay in instrument modification, no progress has been made on the

Scanning electron micrograph of the surface of NHBE cells. Ciliated (arrow) and non-ciliated cells with surface microvilli are apparent (original magnification: 2000X).
third objective, which was to develop SPME procedures for the detection of geosmin and 2-methylisoborneol in water. Both compounds contribute to the problems of off-flavors in catfish even when present in the water at low concentrations.

The use of SPME and GC/MS is anticipated to improve the ability to detect zinc phosphide and metaldehyde in clinical cases of poisoning by these compounds. The use of SPME for the detection of geosmin and 2-methylisoborneol should improve the ability to detect these compounds in pond water for catfish. This methodology will assist in collaborative research into controlling the problem of off-flavors in catfish, which currently relies on detection by the human nose. It is anticipated that both the second and third objectives in this project will be accomplished by August 2001.

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Research Funding

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*Excluding carryover funds

Georgia Livestock and Poultry: Inventories and Values

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*Based in part on information published by the Georgia Agricultural Statistics Service, Athens, Georgia
*bIncludes value to dairy cattle and milk produced

Georgia Farm Cash Receipts

<table>
<thead>
<tr>
<th>Category</th>
<th>Percent of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry and Eggs</td>
<td>51.6</td>
</tr>
<tr>
<td>Crops</td>
<td>36.4</td>
</tr>
<tr>
<td>Meat Products</td>
<td>7.0</td>
</tr>
<tr>
<td>Dairy Products</td>
<td>4.0</td>
</tr>
<tr>
<td>Other Livestock</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Note: numbers in parentheses are percent of total farm receipts

Budsberg, S.C. Investigate arthritic disease in dogs. Anon- 

mous, $352,248.


Coffield, J.A. Mechanism of botulinum toxin action. Thomas Jefferson University, $18,915.

Neuromuscular targets of botulinum toxin. National Institutes of Health, $139,183.

Crowell-Davis, S.L. Canine behavior problems. Anon-

mous, $30,471.


Dickerson, H.W. Can a DNA vaccine induce cutaneous immunity in fish? Cornell University, $24,660.

Research training experience for veterinary medical students. Merck Company Foundation, $30,000.

Evans, D.L. Streptococcus iniae infection in trout and tilapia: Host-pathogen interactions, the immune response towards the pathogen and vaccine formulation. BARD, $143,000.

Finco, D.R. The ability to induce chronic anemia in the cat nephrectomy model after phlebotomy. Pfizer Inc., $19,756.


To determine if cats that undergo renal ablation and nephrectomy develop chronic anemia. Pfizer Inc., $56,624.


Induced anemia in the cat nephrectomy model. Pfizer Inc., $43,100.

To evaluate the efficacy of erythropoetin gene therapy product in cats. Pfizer Inc., $111,486.


Fu, Z. Development of recombinant rabies virus vaccines for animals. American Home Products, $70,000.

Garcia, M. Targeted molecular typing of pathogenic avian mycoplasmas. BARD, $113,000.

Characterization of infectious laryngotracheitis virus (ILTV) vaccine derived viral sub-populations. U.S. Poultry and Egg Association, $45,540.

Identification and characterization of retroviral insertion in fowlpox laryngotracheitis viruses. U.S. Department of Agriculture, $34,146.

Hoenig, M.E. Diabetes and impaired glucose tolerance. Pfizer Limited, $56,760.

Hofacre, C.L. Reducing breeder house salmonella, other pathogens, and dust using an electrostatic space charge system. U.S. Poultry and Egg Association, $71,875.

Howarth, E. W. Uromoniiasis in Riverbanks Zoological Park fishes: Diagnosis and isolation of the causative agent. Riverbanks Zoological Park, $1,000.


Latimer, K.S. Marine resources utilization in Georgia. U.S Department of Commerce, $39,155.

Lee, M.D. The NARMS Campylobacter jejuni culture procedures may be selecting for antibiotic-resistant strains. U.S. Poultry and Egg Association, $28,965.


Li, W.O. Characterization and isolation of proliferation-stimulating components of an extract from lactobacillus. Ye Cherng Industrial Products Co., $38,530.

Little, S.E. Efficacy of Heardgard-30 chewables when administered to dogs 120 days after challenge with infective Dirofilaria immitis larvae. Merial Limited, $7,177.

Lukert, P.D. Development of experimental challenge models for bovine enteric disease (E. coli, rotavirus, and coronavirus) in newborn calves and testing of candidate vaccines by challenge studies in newborn calves. Merial, $50,000.


McCall, J.W. Antifilarial drug evaluation in dogs. World Health Organization, $60,000.

Supply of Brugia infective larvae. National Institutes of Health, $96,487.

Filariasis repository research service. National Institutes of Health, $244,187.

Retroviral transduction and immortalization of filaria. National Institutes of Health, $515,727, University of Alabama, $25,000.


Moore, J.N. New synthetic endotoxin antagonists incorporating structural features of the lipid-A of rhizobium sin-1. American Heart Association, $60,000.


LPS-binding protein and the major endotoxin receptor in horses with colic. Morris Animal Foundation, $26,537.


Opioid receptor subtype affinities of novel analogs. University of Maryland, $1,800.

Nettles, V.E. Southeastern cooperative wildlife disease study. Southeastern States, $196,420.


Quist, C.F. Development of scientific information on the animal traps for selected wild vertebrates species by providing necropsy data on injuries associated with the use of animal restraint devices. U. S. Department of Agriculture, $29,889.


Immunological characterization of skin from the Atlantic bottlenose dolphin (Tursiops truncatus). U.S. Department of the Navy, $6,213.

Sanderson, S. A placebo controlled, cross-over clinical study evaluating plasma carnitine concentrations and L-carnitine supplementation of glycemic control and triglyceride levels in dogs with diabetes mellitus. Ralston Purina Company, $37,827.


Adaptation and attenuation of infectious bronchitis virus. Merial Select, $75,565.


Woolums, A.R. Multidisciplinary evaluation of fatal feedlot ARDS. U.S. Department of Agriculture, $240,000.
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