Center for Veterinary Medicine (CVM)

Three-Year Research Plan

FY2009 – FY2011

Letter from the Office of Research's Director

The Office of Research's Mission Statement states our commitment to conduct applied research in support of FDA regulatory issues and ensure the safety of animal derived food and animal health products. Another equally important goal is to involve our customers. The main goal of this document is to strengthen the relationship that we have with our customers by seeking their help in developing this and future three-year plans.

The staff of the Office of Research (OR) is committed to providing quality science to address regulatory issues of the Center for Veterinary Medicine (CVM) and the Agency. To increase customer involvement, the OR Leadership Team is formalizing the process of prioritizing the Center's research programs. This 2008 CVM Three-Year Research Plan initiates the Center's research planning process for FY 2009. This formal process will inform our customers of research plans for the next three years and serve as the resource base for subsequent research plans. The process will also provide our customers with the ability to establish research priorities and be involved in research plan approval.

While the CVM Three-Year Research Plan represents the culmination of the annual planning process, the annual CVM Research Report is another component of the planning process. This report is designed to provide our view on progress and accomplishments. This report also provides our customers and management the opportunity to view progress in terms of priorities for competing resources. Certainly, as stewards of the resources provided, we must continually view our work from the perspective of value added to the safety of the products we regulate.

The OR is committed to its mission of providing research and scientific expertise to the Center and Agency. The OR staff, with your input in establishing priorities, is focused on our mission and the value our work brings to the safety of the United States food supply. We are excited about our research programs and the opportunity we have for contributing to public health. We encourage your involvement.

Marleen Wekell, Ph.D.
Director
Office of Research

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The Three-Year Plan

The Center for Veterinary Medicine (CVM) Three-Year Research Plan describes the proposed research programs of the Office of Research (OR). This research plan is designed to meet the needs of CVM over the next three years. CVM promotes both public health and animal health through the approval of new animal drugs and feed additives and the post-approval monitoring of the use of these products. The main offices of CVM include the following groups who are both major OR customers and stakeholders: Office of New Animal Drug Evaluation (ONADE) and Office of Surveillance and Compliance (OS&C). OR also accepts assignments from the Director of CVM and collaborates with other organizations and researchers on issues related to CVM's mission and initiatives.

The following sections describe impacts made by OR research, CVM goals to be fulfilled, and long-term benefits that CVM will receive with this proposed Research Plan.

Impacts of CVM Research

The following list describes how OR's research has positively impacted CVM and FDA regulatory programs.

- OR scientists evaluated three commercially available test kits used to detect prohibited proteins in animal feeds. These evaluations provided the Agency with information regarding the accuracy and usefulness of such tests in support of the Feed Ban.
- OR scientists tested the antimicrobial susceptibility of over 200 strains of *Aeromonas salmonicida,* providing information to determine epidemiologic cutoff values for this important fish pathogen. These data will help the Agency when it begins the process of determining clinical breakpoints for antimicrobials used to treat this disease.
- OR scientists developed an HPLC method to detect oxytetracycline in fish serum. Such methods are needed to determine the concentrations of drug that can be reached using a proposed dose and thus help determine if the drug would be clinically effective for specific organisms with known MIC values. The Agency uses such information when deciding what MIC levels to include on drug labels.
- OR scientists provided incurred residue tissues to develop methods to identify melamine and cyanuric acid in fish, swine and chicken tissues. In addition, they provided crucial information to the agency on the development of renal crystals as a result of combined administration of those two compounds.
- OR scientists published the first description of a laboratory transmission of an internal monogenean in fish. This disease model can be used to define methods to run efficacy drug trials for parasitic infections in fish.
- OR scientists have developed new multiclass drug residue screening methods that can simultaneously detect and differentiate between 20 to 40 different compounds in eggs, fish and shrimp. These methods use the specificity and sensitivity of liquid chromatography-tandem mass spectrometry (LC-MS/MS) combined with modern computing techniques to accomplish this feat.
- Building on the multiclass drug residue method in fish, OR scientists developed an LC-MS/MS method for erythromycin and its metabolites in fish. This chemical method provided greater selectivity than the microbiological inhibition method that was provided in support of the NADA. The chemical method was bridged to the micro method using tissue from erythromycin-dosed fish.
- OR scientists have developed standardized testing methods for the *in vitro* antimicrobial susceptibility testing of aquatic microorganisms, Group 1 species. These methods include both disk diffusion and broth microdilution techniques. These testing methods have recently been published by the Clinical and Laboratory Standards Institute (CLSI- formerly the National Committee for Clinical Laboratory Standards, NCCLS). These methods are being adopted by aquatic laboratories world-wide.
- OR scientists provided the pivotal effectiveness study for the use of formalin in rainbow trout. The data filled a critical gap in this important MUMS project.
- OR scientists, responding to urgent Agency requests, developed a method for confirming residues of nitrofurans, banned animal drugs, in shrimp and transferred this technique to an ORA laboratory. Nitrofurans are antimicrobial agents that are banned for use in food animals
- OR scientists coordinated the NARMS testing of retail meats, which supports FDA's surveillance activities geared to monitor the safe use of antimicrobials in food animals. This study allows CDC and CVM to monitor the antibiotic susceptibility profiles of foodborne bacterial pathogens

at the point of purchase by the consumer. The results from this study, in combination with those data from USDA and CDC, will serve as an early warning system for resistance should foodborne bacterial pathogens begin to show decreased susceptibility to various antibiotics being used in animal husbandry and human clinical medicine.

- OR scientists completed testing of over 150 animal feeds for antimicrobial resistant *E. coli* and *Enterococcus*. These data provide baseline data for the microbial status of animal feeds as it relates to antimicrobial resistance.
- OR scientists improved and validated a polymerase chain reaction (PCR) method for the detection of rendered bovine-derived materials in animal fees. The validation of this method provides an important tool for enforcing the Agency's ban on feeding cattle certain types of animal-derived protein. This methodology was transferred to ORA laboratories for use as a confirmatory procedure for the official feed microscopy method.
- OR scientists, working with collaborators at the Florida Department of Agriculture and ORA, validated methods for the screening and confirmation of chloramphenicol residues in shrimp. This drug is known to cause aplastic anemia in susceptible people.
- OR scientists began development of a standardized disk diffusion method to test *Campylobacter* species for resistance to 4 antimicrobials. Such a standard will provide a simple and inexpensive method for routine susceptibility testing of these organisms for surveillance purposes, as well as routine susceptibility testing in the clinical setting.
- OR scientists compiled a pharmacokinetic literature database containing information from over 350 articles regarding drug and chemical metabolism in over 100 different fish species. This resource provides a wealth of rapidly retrievable information to CVM reviewers of drug applications, as well as those trying to conduct minor species drug approval studies.
- OR scientists determined that a commercial milk test kit for beta-lactam drugs did not perform consistently. These data provided the basis for OS&C to issue a warning letter to the firm. As a result, only test kits meeting independent laboratory lot release testing can be sold in the US.
- OR scientists have built up the largest PFGE DNA fingerprinting database of *Campylobacter* in the US, and shared the database with the national molecular subtyping network for food borne disease surveillance program-PulseNet. The national DNA fingerprinting plays a significant rule in detecting and tracing the source of foodborne illness outbreaks.
- Data generated from the National Antimicrobial Resistance Monitoring System (NARMS) provide information for use by sponsors and CVM in the preapproval review process of new animal drugs as outlined in Guidance for Industry #152.

Maintains a Strong Science Base

The Three-Year Research Plan ensures that CVM maintains a productive research program. This enhances OR's ability to recruit and retain scientists with the appropriate scientific base. Involvement with state-of-the-art techniques and current problems provides CVM scientists with the necessary scientific competencies to effectively interact with their industry and academic counterparts. It also makes it easier for CVM to recognize shifts in the technology and scientific fields that regulated industry relies on for future products.

Resolves Cutting Edge Issues

The Research Plan includes both *intramural* and *extramural* components. When expertise on a cutting edge issue resides outside of OR, we fund and monitor these extramural studies when funding is available. However, if one of the following criteria is met, importance, timeliness, or responsibility, intramural research is conducted. Intramural research provides OR with the ability to rapidly develop and conduct research studies in response to pressing needs. This is one of the strengths of OR's research program.

For example, a research question or issue may be so important that CVM chooses to conduct intramural research even though others may have reported results or are actively pursuing a problem. For these issues, where a regulatory decision or policy change will occur, CVM independently confirms new findings. Often, because of regulatory deadlines or public health emergencies, there is not sufficient time to make use of grants, contracts, or cooperative agreements with academic or industrial researchers. The intramural program can change priorities rapidly to facilitate such research. Intramural research is also conducted if no one sponsor may be legally responsible to address the issue or if an industry is too small, e.g., minor species producers or animal diagnostic tests, to support a research effort and address the concerns of CVM.

Answers CVM Needs

The Three-Year Plan includes research designed to answer some of CVM's immediate needs:

- Develop and validate test methodologies, including methods for newly prohibited drugs such as antiviral drugs in poultry.
- Survey drug residues in animal-derived foods.
- Evaluate animal diagnostic tests.
- Monitor changes in antimicrobial susceptibility profiles of selected foodborne bacterial pathogens

On a slightly longer time horizon, a number of research programs will help CVM develop policy or produce guidelines for industry. These programs are described in section 1 and include:

- Effects of CVM approved drugs on efficacy and animal safety
- Immunopharmacology
- Metabolism and residue depletion
- Pharmacokinetics

Provides Microbiogical Food Safety Research

The FDA is mandated to ensure the microbiological safety of foods, including those derived from animals. CVM's research program identifies and investigates microbiological hazards associated with food produced by animal agriculture. Even though the American food supply is among the safest in the world, the program's goal is to further reduce the incidence of foodborne disease to the greatest extent possible.

The role of CVM in this research relates to microbial hazards associated with pre-harvest phases of the animal production environment, including animal feeds. The Research Plan includes research programs to resolve these issues:

- Evaluate animal feeds for the presence of human foodborne bacterial pathogens.
- Identify factors associated with the presence and persistence of zoonotic bacterial pathogens in the animal production ecosystem.
- Survey various food products for the presence of zoonotic foodborne bacterial pathogens.
- Apply genetic typing methods to track the spread of specific zoonotic foodborne bacterial pathogens.
- Identify and compare antimicrobial resistance genes in foodborne pathogens isolated from different sources to better understand the spread of resistant bactiera in the food chain.

Collaborative Research

We in the Office of Research (OR) believe that it is extremely important to be involved in collaborative research and outreach programs. These programs help us to make better use of our resources and facilities. They also provide us with a more diverse viewpoint of how best to accomplish our objectives. The collaborative research programs that we are currently supporting include both formal and informal arrangements, as described in the following sections.

Formal Arrangements

Formal collaborative research programs include:

- **Analytical Methods Development Contract** - This contract was funded to help the Center conduct method trials for new and improved analytical methods for animal drug residues.
- **Joint Institute for Food Safety and Applied Nutrition (JIFSAN)** – CVM/OR collaborated with the University of Maryland on food safety projects funded by JIFSAN including a project (1) to evaluate the *in vitro* metabolic profiles as a method to predict residue depletion of drugs in different species of fish
- **University of Maryland Contract** – CVM has provided funds to examine the prevalence of and genetic relatedness of *Enterococcus* samples from retail meats and humans.
- **Office of Science & Health Coordination (OSHC) (FDA Collaborative Science Project Application)** – OSHC funds CVM/OR's collaborative effort with FDA/CDRH scientists to evaluate the safety and effectiveness of drug-device combinations for treatment of vascular disease. CVM/OR's contribution is to perform the analytical chemistry to determine by liquid chromatography-mass spectrometry the drug levels in blood and vascular tissues resulting from device implantation.
- **USDA Interagency Agreement** – CVM and USDA, Science and Technology Office and the USDA Monitoring Programs Office are collaborating on the determination of antimicrobial susceptibility profiles and generic relatedness of *E coli, Salmonella* and *Shigella* isolates recovered from the USDA-AMS produce surveys. The ultimate objective is to collect data that will be used to establish "benchmarks" by which to evaluate the efficacy of procedures to reduce or eliminate harmful food borne microorganisms.
- **Memorandum of Understanding** – CVM and CFSAN have established an MOU with the Central Sciences Laboratory of the United Kingdom to provide for the cooperative exchange of scientific expertise, assistance, and information to enhance our capabilities to carry out our responsibilities in consumer protection and public health with regard to issues of food safety.
- **Critical Path Initiative** — Interrogating *Salmonella* Diversity Using a Novel 35 Genome *Salmonella* Species Microarray – The FDA Critical Path Initiative awarded funds to CVM and CFSAN to investigate *Salmonella enterica* serotypes of importance to food safety for antimicrobial resistance genes, virulence genes, mobile genetic elements, single nucleotide polymorphisms, and evolutionary relatedness using a newly developed and highly dense affymetrix microarray platform. The goal of this study is to generate data to provide information to increase timeliness and specificity of public health monitoring of FDA regulated food and animal feed products.

Informal Arrangements

Informal collaborative research programs include:

- **AOAC International (**formerly **Association of Official Analytical Chemists)** - This collaborative effort involved the co-development and use of standardized test kit validation protocols for the evaluation of screening test systems used in the detection of drug residues in fresh bulk tank milk samples.
- **University of Maryland** – (1) This informal collaboration provided for the study of the prevalence and antibiotic resistance profiles of food pathogens from conventional and organic production facilities. (2) Completed a joint project to evaluate and potential of the food supply to serve as a reservoir of *Enterococcus* infecting humans. (3) Began a trial to determine the prevalence of methicillin-resistant *Staphylococcus aureus* in retail raw meats. (4) Began a joint project to identify the carriage of virulence factors in E. coli from retail raw meats.
- **University of Massachusetts** – This collaboration will provide for the development of fish models for testing veterinary drugs against major aquaculture bacterial diseases.
- **University of Maryland/USDA/CFSAN/J. Craig Ventner Institute ─** Completed a study to sequence the genomes of 17 *Salmonella* strains, representing 12 serotypes. Reported the complete DNA sequence of a large multi-drug resistance plasmid from *Salmonella enterica* serotype Newport and its distribution in enteric bacteria in retail meats.
- **PulseNet** – This collaborative effort was initiated by CDC and involves State Departments of Health and FDA and USDA. It is an epidemiological database focused on the acquisition and storage of DNA fingerprints of bacterial pathogens generated by the procedure known as pulsed-field gel electrophoresis. This database represents a powerful epidemiological tool to conduct trace-back investigation during outbreaks of foodborne illness leading to faster intervention and establishment of control measures. The food borne bacterial pathogens subtyped by OR's laboratory are obtained from ten FoodNet sites and six state veterinary diagnostic laboratories.
- **Invitrogen -** The goal of this collaborative project is to develop and validate test kits for use with Real-Time PCR in the detection of plant and animal proteins.
- **Consortium for the Barcode of Life –** This project will focus on the development of DNA barcoding systems that will revolutionize taxonomy by enabling prompt and reliable identification of species regardless of the life stage examined or the condition of the specimen.
- **Dr. Mussaret Zaidi, Dept de Investigacion, Hospital General O'Horan, Merida, Yucatan, Mexico** – Completed a collaborative project to characterize resistance among salmonellae and initiated a joint project to characterize resistance among *E. coli* and *Campylobacter* from humans, animals and retail meats in Mexico.
- **USDA –** IAG to measure antimicrobial susceptibility of food borne pathogens isolated from produce via the Agricultural Marketing Service's program.
- **Summer Student Intern Program** – This is a special program for minority college students which gives them experience in biological and chemical research.
- **CFSAN/UMD** – This collaborative effort is to use six –enzyme pulsed-field gel electrophoresis (PFGE) analysis of *S*. Typhimurium and *S*. Enteritidis to determine phylogenetic and taxonomic accuracy of the strain relationships. The plasmid-mediated antimicrobial resistance among these strains will be investigated.
- **Max Plank Institute** – This project compares *Salmonella enterica* serotype Newport isolates from similar time periods from Germany and the United States by MLST type and antimicrobial susceptibility profile and investigates the development of the multidrug phenotype MDR of this

serotype. This work is performed in collaboration with Dr. Mark Achtman,at the Max Plank Institute in Berlin, Germany, curator of the international *Salmonella* MLST database.

- **Ohio State University/North Carolina State University** – Continued a project to compare microarray, and other strain typing methods, for characterizing *Campylobacter jejuni* and *C. coli* isolates obtained from the NARMS program and the animal production environment.
- **Washington State University –** OR scientists are participating in a multi-lab validation of a microarray chip developed by Dr. Margaret Davis at Washington State University identifying antimicrobial resistance genes in bacteria of veterinary importance.
- **University of Minnesota -** The goal of this study is to assess how resistance to various antimicrobials may relate to the virulence potential and phylogenetic background of clinical *Escherichia coli* isolates from retail meats. Also, initiated a collaborative study to investigate the evolution of florfenicol resistance genes in *E. coli* and *Salmonella*.
- **JMI Laboratories** – Completed preliminary studies to develop a disk diffusion testing method for *Campylobacter*.
- **Central Science Laboratory –** This collaboration will be for the joint development and validation of methods to determine and confirm antiviral drug residues in poultry.

Research Prioritization

The CVM Three-Year Research Plan is the final product of an annual review and prioritization of research programs and activities. As stated in the Office of Research Director's letter at the beginning of this document, OR is formalizing this review process that includes input from OR scientists and managers, the other offices in CVM, external stakeholders, and CVM's Senior Management Team (SMT).

The following figure summarizes the annual review and selection/prioritization of research activities.

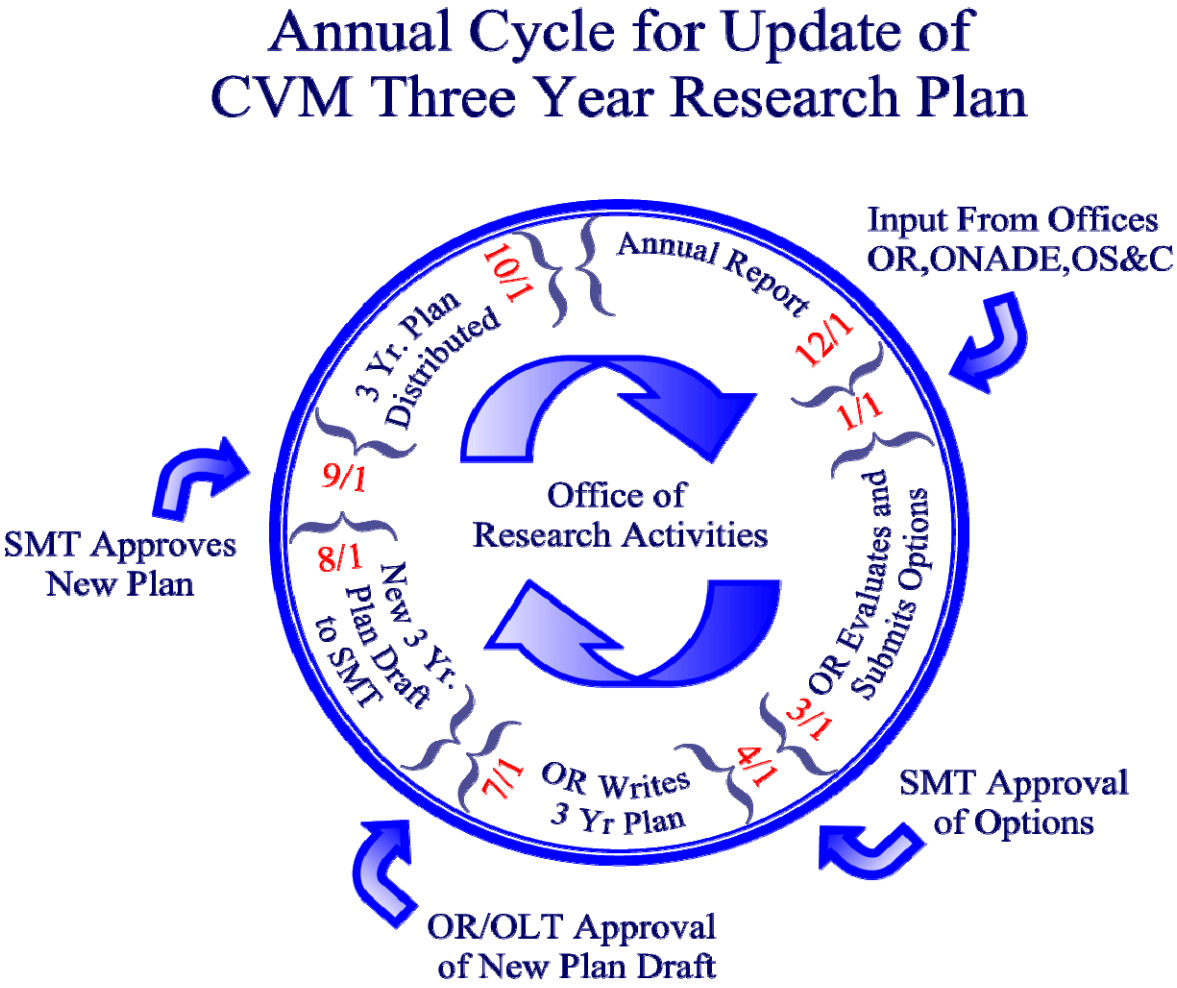

Figure 1. Selecting and Prioritizing Research Activities

In December of each year, the Office of the Center Director, the Offices of New Animal Drug Evaluation and Surveillance and Compliance, and external stakeholders are requested to assess the current Research Plan. They determine which activities should continue, and which areas need redirecting. Secondly, they identify and prioritize their own research needs. Typically, these needs include:

- Data to support guideline development
- Product investigations
- Generic data gaps
- Feasibility studies
- Surveys
- Method development

The next step in the prioritization process is for OR scientists and management to review these requests and comments. Requests are sorted into those that either can or cannot be accomplished with OR's expertise, resources, and facility. For those requests that cannot be accomplished at OR, we provide suggestions (cooperative agreements, contracts, etc.) and estimated costs for meeting them. Those requests that we believe OR can address are evaluated in terms of the necessary resources and time. From this, OR develops multiple options for the revision of the plan.

These options and their associated costs are presented to the SMT in April. They review the current plan and the options presented for update and revision. They select, modify, and approve various proposals.

OR uses this guidance to revise the Three-Year Plan and submits the updated plan to the SMT for its approval in August. Upon approval by the SMT, the updated Three Year-Plan is released and is effective October 1, the start of the new fiscal year.

Also at this time, OR scientists and management prepare the annual report of research activities of the prior fiscal year. This is distributed by December 1 so that the other offices in CVM and external stakeholders can consider it in formulating their proposals in the next cycle of research prioritization.

Document Organization

The CVM Three-Year Research Plan is organized into four sections. These groupings of research recognize the connection between research and the respective pre- or post-approval activities of the Center. They are also the four core components of the Center's "Back to Basics" strategic initiative.

- Premarket/ Drug Review
- Compliance
- Post-Approval Monitoring
- Animal Feed Safety

Each section introduces the category of research, and then describes the active research programs, the participating researchers and organizations in each program, the resource commitments, and the objectives for the next three years.

Abbreviations Used in This Document

The following table presents a list of abbreviations that appear in this document.

Abbreviation	Description
AOAC	Formerly Association of Official Analytical Chemists
ARS	Agricultural Research Service
AST	Antimicrobial susceptibility testing
BAM	Biological Analytical Manual
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CFSAN	Center for Food Safety and Applied Nutrition
CLSI	Clinical and Laboratory Standards Institute (Formerly NCCLS)
CVM	Center for Veterinary Medicine
CYP	Cytochrome P450
DAFM	Division of Animal and Food Microbiolgy
DNR	Department of Natural Resources
FDA	Food and Drug Administration
FSIS	Food Safety and Inspection Service
FTE	Full time equivalent
FY	Fiscal year
GLP	Good laboratory practices
HAB	Harmful Algal Blooms
HACCP	Hazard analysis and critical control points
IACUC	Institutional animal care and use committee
IND	Investigational new drug
JIFSAN	Joint Institute for Food Safety
NADA	New animal drug application

Abbreviation	Description
NCCLS	National Committee for Clinical Laboratory Standards
NOH	Notice of Hearing
OLT	Office Leadership Team
ONADE	Office of New Animal Drug Evaluation
OR	Office of Research
ORA	Office of Regulatory Affairs
OS&C	Office of Surveillance and Compliance
PAG	Project Advisory Group
PCR	Polymerase chain reaction
PD	Pharmacodynamics
PI	Principal Investigator
PK	Pharmacokinetics
PO	Project officer
SMT	Senior Management Team
SOP	Standard operating procedure
UMB	University of Maryland at Baltimore
USDA	United States Department of Agriculture

1 PREMARKET/DRUG REVIEW

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Introduction

The mission statement for Agency research states "FDA uses innovative science-based decision-making to efficiently evaluate and make available beneficial new products, to develop sound safety standards and guidance, and to rapidly identify and address emerging public health needs." Research programs at CVM's Office of Research follow the direction set by the Agency by enabling the Center to resolve regulatory issues on a scientific plane based on principles and data. Resolution of these issues sometimes takes the form of guidance documents or Center policies. These documents and policies direct the regulated industry in the drug approval process or in the proper use of drugs and feed additives.

The Premarket/Drug Review research program ensures that these guidelines and policies are based on sound science, as in 1996 when the Animal Drug Availability Act (ADAA) modified a section of the Food, Drug, and Cosmetic Act. The section pertained to how the regulatory standard for demonstrating drug effectiveness was defined. Congress amended the definition due to substantial evidence. The amended definition permitted the FDA more flexibility in determining the types of studies required to demonstrate that a particular new animal drug was effective for its intended uses and conditions of use.

Current OR research programs will help the Center develop policies or guidelines that allow for this flexible approach to demonstrate efficacy. Research programs for the Three-Year Plan include:

- **Animal Drug Safety and Efficacy** – We are supporting the availability of new aquaculture drugs by performing safety and efficacy studies.
- **Antimicrobial Resistance Mechanisms** – As part of our food safety programs, OR scientists are evaluating issues related to the use of antimicrobial drugs in both humans and animals to develop new animal drug requirements and policies that protect the public health.
- **Immunopharmacology** – Efforts are underway to identify biomarkers of disease within the immune system that could demonstrate the effectiveness of drugs.
- **Metabolism and Residue Depletion** – To aid in the review of new aquaculture drugs, we are examining the comparative metabolism and depletion of select veterinary drugs in several species of fish.
- **Method Development** – OR scientists are developing regulatory methods in support of minor species drugs and investigating new approaches to regulatory methods.
- **Method Trials** – OR scientists participate in the laboratory evaluation of regulatory methods submitted in NADAs.
- **Microbiological Methods** – To ensure uniform and reproducible data on microbiological safety in submissions, OR scientists are developing standardized antimicrobial susceptibility test methods.
- **Pharmacokinetics/pharmacodynamics** – Recent investigations compared the pharmacokinetics of drugs in two production classes of animals in the same species and the bioavailability of drug formulations across species.

Animal Drug Safety and Efficacy

FDA uses innovative science-based decision-making to efficiently evaluate and make available beneficial new products, to develop sound safety standards and guidance, and to rapidly identify and address emerging public health needs. Research programs at CVM's Office of Research follow the direction set by the Agency by enabling the Center to resolve regulatory issues on a scientific plane based on principles and data. Resolution of these issues sometimes take the form of guidance documents or Center policies to direct the regulated industry in the drug approval process or in the proper use of drugs and feed additivies. It is imperative that these guidelines and policies be based on sound science.

In addition, we have been asked to conduct studies to provide pivotal data to support public master files of selected, potential drugs for aquatic species. We are developing protocols for efficacy and safety studies. We have developed a fungal infection model in catfish to facilitate anti-mycotic drug efficacy studies and a bacterial infection model for trout *A. salmonicida,* an important aquaculture pathogen. This latter model will help us develop criteria with which to judge the potential effectiveness *in vivo* of antimicrobials showing *in vitro* effectiveness against this pathogen. We have recently published a fish model for internal parasite infection in which to test the efficacy of anthelmintic drugs. This work will help support the Center's goal to increase minor species drug availability.

Test Anthelmintics in Largemouth Bass Infected with *Acolpenteron ureteroecetes*

Acolpenteron ureteroecetes is a monogenetic trematode, a that parasite requires only one host in its life cycle. As intensive aquaculture of largemouth bass is increasing, this parasite has been reported as an emerging disease, especially in recirculating culture systems At this time, there is no approved effective treatment for these internal trematodes, in fact there are no FDA approved treatments for any internal parasites of fish.

Since parasites are a major source of fish mortalities and aquaculture production loss, there is a need for effective theraputic agents. However, CVM has no guidance for industry to aid investigators trying to conduct aquatic parasite efficacy studies.

The ultimate purpose of the current project is to develop specific test methods to evaluate the effectiveness of parasiticides on infected fish. By conducting such work at CVM we will be able to provide insight into design aspects of such studies in aquatic species. This information is very valuable to obtain for reviewers in the Aquaculture Drug Team in ONADE as they are frequently called upon to comment on study design by drug sponsors.

We will increase our stocks of infected animals to conduct this study since infected fish have a much higher mortality rate than uninfected fish. Such studies require maintaining a colony of infected animals at CVM, a rare recourse for such investigations.

We are in the process of evlauating extensive video microscopic data to determine drug candidates that will be used for the *in vivo* part of the study. Preliminary data indicate several drugs have effect on motility and survival of these trematodes. Effectiveness within the target organ (kidney) must, however, be evaluated in the living fish. Questions to be addressed in our study will be – What is the level of ambient infection (prevalence and intensity) necessary to demonstrate clear response post treatment? What dosages show response? What criteria for efficacy are acceptable if the parasite cannot be completely eradicated in a fish population?

The following personnel are responsible for this study:

- Renate Reimschuessel
- Charles Gieseker
- Eric Evans
- OR staff

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Analyze video images of parasite responses to anthelminics *in vitro* to determine potential candidate drugs for in vivo testing. Obtain and infect more large mouth bass juveniles.	0.3
10	Develop protocol for *in vivo* testing of *A. ureterectes* as a model for parasite efficacy studies for internal parasites. Begin effectiveness study using candidate drugs based on *in vitro* data.	0.3
11	Complete effectiveness study using candidate drugs based on i*n vitro* data	0.2

Examine Effectiveness of Formalin Treatment of Fungal Disease in Catfish

Aquatic fungi (*Saprolegniales*) are ubiquitous in natural water supplies of fish hatcheries and can cause serious disease problems. *Saprolegnia* infections (saprolegniasis) in fish are promoted by poor water quality, malnutrition, injuries occurring during handling, crowding, temperature shock, spawning, and external parasitism. Saprolegniasis can also occur as a secondary infection to existing bacterial or viral infections. The resulting infections on fish generally occur to the epidermis, dermis, and possibly the superficial musculature causing osmoregulatory failure.

Some preliminary studies and hatchery trials with formalin show promise that formalin could be effective in preventing or controlling mortalities associated with saprolegniasis in fish. Definitive controlled studies are needed, though, to clearly define efficacy and determine optimal treatment regimes. We completed studies in rainbow trout, which have been accepted by ONADE in support of a publically sponsored INAD. We were asked to provide similar information for channel catfish to compare with the trout data. We have completed the laboratory phase of this GLP study. Once the data has undergone QA/QC review, we will write a final report, which will be submitted to ONADE. This study will provide pivotal data on the efficacy of formalin to treat fungal infections in fish.

Recently ONADE has requested that our laboratory also evaluate the appropriateness of a Fish and Wildlife Service (UMESC) fungal infection model being used to evaluate other therapeutants. Our scientists will collaborate with UMESC scientists to compare the 2 infection models.

The following personnel are responsible for this study:

- Renate Reimschuessel
- Charles Gieseker
- Eric Evans
- OR staff
- Joan Gotthardt, CVM/MUMS
- Joan Gotthardt, CVM/ONADE

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Complete QA/QC of GLP study and prepare final report	0.2
10	Compare UMESC disease model with CVM model	0.6

Develop Criteria for Establishing Aquatic Animal Disease Models

Clinical efficacy trials of natural disease outbreaks are very difficult to complete in aquatic species. This is due to several factors, including the rapidity that many disease outbreaks progress to death, the fact that most hatcheries are ill equipped to run well controlled clinical trials and the difficulties in documenting the disease status of individual animals or their consumption/exposure to the drug.

Increasingly, there is a need to develop well controlled disease models for aquatic animal drug efficacy trials, which do not suffer from the limitations found in natural outbreaks. There are, however, many variables which need to be defined when developing a disease model. For example, what routes are acceptable for infecting the animals, what percent of exposed animals should become ill, is it necessary (and/or preferable) for the disease to be created in 100% of the animals, how severe should the disease be (i.e. what percent mortality in untreated controls), how soon after the disease has been diagnosed should the treatment be initiated. These are just some of the questions that arise and as sponsors begin to develop models, they will come to CVM for guidance on these and other points.

The goal of this project is to begin to develop criteria that can be used for developing a CVM guidance document for developing aquatic animal disease models. It is not one specific model we will develop, but to define the method of determining appropriate infection rates, infection severities, time frames and treatment options within a disease model so that the model provides a true means of predicting clinical outcomes in natural infections. As a first model, we will work on parasite enumeration and treatement evaluation.

Additional questions to be addressed in following stages of this project are an examination of Gyrodactylid and Dactylogyrus species to determine if all species can be grouped together with the expectation that treatement response within genus will be consistent.

The following personnel are responsible for this study:

- Renate Reimschuessel
- Charles Gieseker
- Eric Evans
- OR staff
- Don Prater, CVM/ONADE

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	*Develop Protocol* for defining specific criteria of concern in collaboration with ONADE reviewers. Initiate studies to demonstrate methods for obtaining data.	0.3
10	Obtain and infect appropriate fish species and parasites as identified by ONADE and OR Evaluate genus vs species level response to treatments	0.5
11	Conduct effectiveness study using model drugs	0.5

Examine Depletion and Crystal Formation in Fish Following Administration of Melamine and Cyanuric Acid

Melamine, cyanuric acid and a combination of these compounds recently were found in pet feeds, livestock feeds and fish feeds. There is little-to-no information regarding depletion of these triazines in fish tissues. In May 2007, livestock feeds (hog/chicken/fish) were adultereated with the triazines melamine and cyanuric acid. Thousands of animals had eaten these feeds and producers asked the Agency if they could sell the animals for slaughter. There was an outcry for information regarding how long residues remain in tissues and FDA preformed a risk-assessment using the limited information that was available at that time. There was no data about whether residues form in hogs, chickens or fish, nor were there any methods to measure residues in their edible tissues. CVM scientists conducted a rapid study to provide incurred samples for developing methods in fish tissues, and demonstrated that feeding both compounds caused crystal formation in fish kidneys, similar to those that caused renal failure in cats and dogs.

Methods for melamine and cyanuric acid have now been validated, but many questions about how extensively fish accumulate these compounds, residue depletion and no-effect levels remain. One goal of this research program is to determine what residues can be expected when fish consume relatively low doses of these compounds. We will determine depletion rates for these triazines in two species of fish, a warmwater and a coldwater species since depletion rates can vary depending on temperature. In addition, we will determine the threshold dose for development of crystals in fish following triazine administration. Finally we will the effects of sequential administration of individual chemicals.

The data may be used by FDA in conducting risk assessments and will help other centers (such as NCTR) as they develop protocols to explore triazine effects in mammals, with the fish serving as a non-mammalian model representing the worst case senario for residue accumulation.

The following personnel are responsible for this study:

- Renate Reimschuessel
- Charles Gieseker
- Eric Evans
- ORAU Fellow

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Complete depletion study in trout and catfish. Prepare preliminary agency report. Conduct sequential dosing studies and threshold dosing studies.	0.8
10	Prepare final report..	0.2

Investigate specific questions for select Aquaculture INAD agents

Aquaculture drugs are unique in that many of the compounds used to treat fish diseases are compounds not traditionally considered therapuetic agents in mammalian medicine. As a result, personnel reviewing data from studies to support a drug claim will not have adequate background information available to make decisions based on sound science.

Diquat is a contact herbicide that produces desiccation and defoliation. It has also been used to treat fish with presumptive external flavobacteriosis and bacterial gill disease (BGD). There is, however, little documention regarding appropriate dosages and duration of treatment.

We will begin to assess this issue by examining the current disease models for BGD and external flavobacteriosis. We will test selected models in our laboratory to find one appropriate for effectiveness trials in catfish. This model will be optimized and then used to evlauate different dosage regimens for diquat.

The following personnel are responsible for this study:

- Renate Reimschuessel
- Charles Gieseker
- Eric Evans
- OR staff
- ONADE reviewers.

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
10	Evaluate infection models for BGD and Flavobacterial disease, obtain appropriate isolates, conduct in vitro tests for susceptiblity to diquat	0.5
11	Optimize a laboratory disease model and dosing regimen for diquat	0.6

Evaluate the Safety of Using the Ear as an Injection Site for Parenteral Drug Delivery in Cattle

The ear has become an increasingly common site for the injection of therapeutic and production drugs in cattle. This primarily is because the problem of extended drug residues at the injection site is avoided, and it is also less likely to result in carcass damage and trimming required at the time of slaughter. The CVM Office of New Animal Drug Evaluation (ONADE) currently has several drug products approved, or under review, that designate the ear pinna as the site for subcutaneous injection. However, field safety studies with some of these drugs have resulted in several reports of acute death following administration. These cases have been attributed to inadvertent injection of the drug into an artery, resulting in back-flush into the cerebral blood supply, leading to embolism and death. While CVM/ONADE has requested that the drugs in question be studied, in order to determine if the adverse events are volume-related or due to physical/chemical properties of the drug/carrier, it has become apparent that few anatomical references exist that describe the blood vessels on the posterior aspect of the bovine ear and the most likely pathway for injected material to access the cerebral circulation. The purpose of the initial study is to perform an anatomical investigation of the blood vessels in and around the bovine ear, which will be completed prior to initiating a study to investigate the drugs in question. The results of this study should: 1) provide a detailed vascular map of the ear region, 2) indicate the feasibility of back-flush into the cerebral blood supply by inadvertent arterial injection, and 3) aid in estimating the approximate injection volume required for back-flush to occur.

The following personnel are responsible for this study:

- Jeffrey Ward
- Jamie Boehmer
- DAR Staff

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Complete anatomical study in steers and consider the need for a drug safety study	0.3

Investigate the Effects of Late-Term Gestation on Blood Levels of Doramectin

The FDA has become aware of recent incidents involving the misuse of the anthelmintic Doramectin (Dectomax®) to treat dairy cattle. Doramectin is not approved for use in female dairy cattle 20 months of age or older. If a lactating cow is exposed to Doramectin, milk from the cow may have detectable residues of the drug for as long as 60 days. Furthermore, the blood levels that the drug achieves in late-gestation heifers (not lactating), as compared to non-pregnant animals, is not known. Because the drug can be legally administered to pregnant heifers, the current study will be undertaken to evaluate the effects of late gestation on the blood levels of Doramectin. Subsequent studies may investigate the effects of first lactation on blood and milk levels of the drug.

The following personnel are responsible for this study:

- Jamie Boehmer
- Jeffrey Ward
- DAR Staff
- Janis Messenheimer, CVM/ONADE

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Initiate study in dairy cows	0.2
10	Complete pharmacokinetic study and prepare final report.	0.4

Develop a Model System to Identify Anti-Inflammatory and Analgesic Drug Cytotoxicy Biomarkers Using Pharmacogenomics

There is a need for biomarkers of damage that can be used to compare toxic responses among species. Toxicogenomics may be the most promising approaches to identifying such markers. Using peripheral blood is of specific interest because blood is easily available. Previous studies have shown that sufficient number of nucleated cells supplying mRNA offers the possibility to use peripheral blood for gene expression studies. After a compound has been delivered, blood cells are exposed to significant concentrations of this target agent because drugs or chemicals are distributed via the blood stream and 'direct' blood functions such as immunomodulation can be studied by gene expression analysis. More intriguing is the search for biomarkers in the blood that are predictive for toxic events in target organs, which cannot regularly be studied by gene expression analysis. Identifying cross-organ biomarkers is the first step in identifying cross-species biomarkers. One class of cross-organ biomarkers consists of genes that are simultaneously altered in both blood and the target organ. Nucleated cells of peripheral blood and the cells of the true target organ must have a similar transcriptional response. Since basic mechanisms of toxicity are described in a variety of organs, it is likely that different cell types should exhibit overlapping gene expression responses to toxic stimuli. Additional classes of biomarkers have been identified by studying pathways instead of single genes; however, the number of genes and their signaling pathways that have been linked to toxicological mechanisms is minimal. Finally, peripheral blood is systemically delivered to almost all organs through the body, allowing communication between blood cells and diverse target or effector cells of the entire organism. Therefore, gene expression in blood cells may respond to changes in other organs.

This study will generate genomic anti-inflammatory and analgesic drug toxicity biomarkers for major and minor species. The goals of this study are to 1) generate pharmacogenomic data sets and identify biomarkers for dexamethasone and acetaminophen toxicity using FDA's ArrayTrack and Gene Logic's BioExpress® System data analysis tools; 2) validate gene sets by RT-PCR; and 3) develop assays to provide molecular toxicity evidence beyond traditional endpoints.

The following personnel are responsible for this study:

- Haile F. Yancy
- DAR staff

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Establish toxicity levels for dexamethasone and acetaminophen in cow blood cultures, , generate toxicogenomics data, identify biomarkers, and validate gene sets by RT-PCR.	1.0
09	Initiate validation of dexamethasone and acetaminophen toxic biomarkers in dog, rat, mouse, chicken, and pig.	1.0
10	Complete validation study.	0.3

Develop Alternative Ivermectin-Sensitive Model Systems – A Critical Path Study

The MDR-1 gene encodes for P-gp, a transmembrane efflux protein that affects the absorption, distribution and elimination of certain drugs. P-gp is a member of the ATP-Binding Cassette (ABC) transport protein family, which are efflux pumps that remove select agents from within the cell. P-gp is part of a family of efflux transporters found along the intestinal tract, in the kidneys, biliary system, brain and other organs. A mutation in the MDR-1 gene is known to occur in several dog breeds. Since P-gp is an important efflux transporter for a wide range of compounds, dogs homozygous recessive for the MDR-1 mutation may have altered pharmacokinetic and toxicity profiles for P-gp substrates including avermectins. Most importantly, life threatening toxicity has been reported when certain P-gp substrates are administered to dogs that are known to be homozygous for the MDR-1 mutation. In addition, there are anecdotal reports of heterozygote animals also expressing toxicity to some P-gp substrates and a small set of homozygous recessive genotype animals do not express the expected sensitive phenotype.

Currently, using Collies is the only avenue of establishing if new drugs can be toxic. Due to a diminishing number of available ivermectin-sensitive Collie colonies, it is becoming difficult for drug sponsors to get the dogs needed to test the safety of new avermectins. Therefore alternative screening procedures are needed to identify potential P-gp substrates and to address safety concerns. This project will develop alternative *in vivo* and *in vitro* models that may be used in lieu of ivermectin-sensitive collies to confirm the safety of new and combination new animal drug products.

The goals of this project are to 1) determine whether the comparative pharmacokinetics of several known P-gp substrates are substantially different when administered to dogs that are homozygous recessive, heterozygous or the wild-types; 2) develop *in vivo* and *in vitro* systems that can be used to identify P-gp substrates; and 3) to validate the new model systems as a suitable replacement for the ivermectin-sensitive Collies.

The following personnel are responsible for this study:

- Haile F. Yancy
- Yolanda Jones
- Nadia Francis (ORAU Fellow)
- Michael Myers

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Determine whether the comparative pharmacokinetics of several known P-gp substrates are substantially different when administered to dogs that are homozygous recessive, heterozygous or the wild-types.	2.5
	Design and create the MDR1 Knock-in/MDR1 beta Knock-out mouse line expressing canine MDR1 mutated cDNA.	0.3
10	Continue creating the MDR1 Knock-in/MDR1 beta Knock-out mouse line expressing canine MDR1 mutated cDNA and begin validation studies.	0.2

Antimicrobial Resistance Mechanisms

Since the introduction of antimicrobials into veterinary medicine some 60 years ago, animal health and food animal productivity have improved significantly. Due to the emergence and dissemination of antibiotic-resistant foodborne bacterial pathogens, there is an increased public and scientific interest in the public health impact of agricultural antimicrobial use.

Bacterial antimicrobial resistance is a serious problem worldwide in both the medical and agricultural fields. Resistance mechanisms have been identified and described for all known antimicrobial agents currently available for clinical use. This situation is being addressed by FDA, AVMA, and USDA who have developed judicious use guidelines and implementing strategies to head off this potential threat to veterinary medicine.

The development of bacterial antimicrobial resistance increases the potential to distribute multiple antimicrobial resistance genes to susceptible bacterial populations. Bacterial antimicrobial resistance usually develops by means of chromosomal mutations or by the acquisition of large, transferable, extrachromosomal DNA elements called plasmids, which may carry many resistance detemrinants. Plasmids may contain additional DNA mobile elements such as transposons and integrons. Integrons contain one or more contiguous resistance genes, acquired as mobile gene cassettes. These cassettes are inserted in various arrangements between two conserved DNA regions, creating tandem arrays of different antimicrobial resistance genes. Over 50 gene cassettes and four distinct classes of integrons have been identified to date. The various DNA mobile elements possess genetic determinants for several different antimicrobial resistance mechanisms, and may be responsible for the rapid dissemination of resistance genes among different bacterial genera and species. In addition to antibiotics, some of these elements contain genes encoding resistance to disinfectants and heavy metals. This raises the possibility that there multiple selection pressures that maintain these linked phenotypes.

As is the case for related bacterial infections in humans, therapeutic options for treatment of some animal diseases are decreasing. It is, therefore, essential that we identify, evaulate, and implement prevention strategies and infection control measures to reduce or eliminate the health hazard posed by antimicrobial resistance. Understanding the molecular basis of antimicrobial resistance gene acquisition and transmission among veterinary bacterial pathogens will help improve antimicrobial treatments strategies and management practices to preserve the potency of antimicrobials for future use.

Determine Microbial Ecology of Antibiotic Resistance Development and Dissemination in the Food Animal Production Environment

Animal production practices rely on the use of antimicrobials to treat and prevent infectious diseases. The extent to which antimicrobial use in animals influences the development of resistance among zoonotic pathogens is unclear. These studies seek to address some of the gaps in our understanding by examining the consequences of antibiotic use in animals on susceptibility changes among the animal's intestinal flora.

The goal of this research is to determine the effects of antimicrobial use in animals on the development of resistant bacteria within the context of a normal production environment. We will first establish the baseline susceptibilities of important endogenous bacteria, then study the emergence and spread of resistance following antimicrobial treatment. In addition, we will study the dissemination and stability of antibiotic resistant organisms once they are established in the environment. This identification of specific organisms involves sampling the animals, their water, excreta, feed, and other possible routes by which these bacteria can enter the environment.

This project will help answer a number of questions using the basic protocol and changing one dependent variable at a time:

- What are the dynamics of resistance in this environment once the drug of choice is removed from the environment?
- Do the resistant organisms persist or do they disappear or diminish in the environment?
- What happens to the resistance determinants after they appear in the environment? Are they transferred to other organisms present? Which organisms? And at what rate?
- How well do these organisms survive in litter/bedding?
- How important is feed as a source of pathogenic or resistant bacteria?
- How does the continuous use of litter/bedding between flocks or herds affect the flora of newly placed animals?
- Is there any intervention strategy that can be used to minimize or eliminate resistance in this environment?
- Can selective pressures reinstate the prevalence of the resistant bacteria?

Many factors must be considered in designing these studies, such as the animal species being treated, the bacterium being targeted, and the antibiotic being administered. The large number of veterinary drugs, some with human analogues, require investigations designed according to the particular usage considerations. These studies address basic questions about the impact of drug use in a normal food-animal production environment, and help establish a framework for designing mitigation strategies to reduce resistance.

The following personnel are responsible for this study:

- Patrick McDermott
- OR Staff

The following table presents the objectives and the title of each study.

FY	OBJECTIVE/STUDY TITLE	FTE
09	Initiate new studies examining the impact of drug use in food animal species	1.0
10	Initiate studies different antimicrobials used in food animal species	1.0
11	Complete laboratory phase of *in vivo* work	1.0

Use Molecular and Biochemical Methods to Determine the Animal Origin of Foodborne Bacterial Pathogens

Bacteria belonging to the genus *Salmonella* cause a majority of the cases of acute bacterial diarrhea in the US and other countries throughout the world. The vast majority of these infections originate in foods of animal origin. This bacterium is part of the normal intestinal microflora of a large number of animals, including those raised for human consumption. Thus, it is not uncommon for foods derived from animals to become contaminated with *Salmonella*. Consequently, improper cooking or handling of animal derived food products can result in human illnesses. These illnesses range from mild self-limiting cases to severe systemic illness that may be fatal. In recent years, 31% of the deaths resulting from bacterial foodborne illnesses have been caused by *Salmonella*.

In order to elucidate possible sources of *Salmonella* attributable to human foodborne disease, phenotypic typing methods such as serotyping, antibiograms, and biochemical profiling have been applied to isolates from animal and environmental sources, as well as clinical strains. Because bacteria from diverse sources may have the same biochemical profile or antibiogram, epidemiological traceback conclusions based on only these typing methods are often tentative. To better identify reservoirs of infection and to strengthen the links between pathogens from various sources, numerous genetic typing methods have been investigated. These methods exploit differences in the DNA base sequence between different strain types. By using a combination of genotypic and phenotypic methods, it has been possible to identify evolutionary clusters of strains and to monitor the spread and persistence of strains within defined environments (e.g., poultry houses). This research program will examine relationships between genetic characteristics (e.g., DNA sequence Multi-Locus Sequence Typing (MLST) and genetic relatedness tree modeling, Multi-Locus Variable Number Tandem Repeat Analysis (MLVA), single and second enzyme pulsed-field gel electrophoresis (PFGE), microarray, and phenotypic characteristics (e.g., serotype, antimicrobial resistance) of *Salmonella* isolates from various sources in order to identify characteristics that will enable epidemiologists to determine the animal of origin of a *Salmonella* isolate associated with a human illness.

Identifying the source of *Salmonella* associated with a specific human illness is a prerequisite for intervention and infection control. The utility of a particular characteristic for typing is related to its stability within a strain and it's diversity within the species. Since reproducibility is one of several criteria in evaluating the usefulness of a typing system, a large number of strains will be examined so that inferences about clonal ancestry can be formed.

The following personnel are responsible for this study:

- Heather Harbottle
- DAFM staff

The following table presents the objectives and the title of each study.

FY	OBJECTIVE/STUDY TITLE	FTE
09	Continue 08 studies. Test other methods for source tracking. Expand the number of strains/serotypes of animal origin to be typed. Expand the study to examine isolates of other *Salmonella* serotypes and other foodborne zoonotic bacteria. Continue development and testing of a DNA microarray platform	1.0
10	Continue the studies with human isolates and compare the results with isolates from food animals. Continue analysis using differentmicroarray designs	1.0
11	Analyze large numbers of strains from various sources using microarray and other methods to detect genetic differences.	1.0

Interrogate *Salmonella* Diversity Using a Novel 35 Genome *Salmonella* Species Microarray – A Critical Path Study

In order to elucidate possible sources of *Salmonella* attributable to human foodborne disease, phenotypic typing methods such as serotyping, antibiograms, and biochemical profiling have been applied to isolates from animal and environmental sources, as well as clinical strains. Because bacteria from diverse sources may have the same biochemical profile or antibiogram, epidemiological traceback conclusions based on only these typing methods are often tentative. To better identify reservoirs of infection and to strengthen the links between pathogens from various sources, numerous genetic typing methods have been investigated. These methods exploit differences in the DNA base sequence between different strain types. By using a combination of genotypic and phenotypic methods, it has been possible to identify evolutionary clusters of strains and to monitor the spread and persistence of strains within defined environments (e.g., poultry houses). This research program will examine relationships between genetic characteristics (e.g., DNA sequence Multi-Locus Sequence Typing (MLST) and genetic relatedness tree modeling, Multi-Locus Variable Number Tandem Repeat Analysis (MLVA), single and second enzyme pulsed-field gel electrophoresis (PFGE), microarray, and phenotypic characteristics (e.g., serotype, antimicrobial resistance) of *Salmonella* isolates from various sources in order to identify characteristics that will enable epidemiologists to determine the animal of origin of a *Salmonella* isolate associated with a human illness.

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The following personnel are responsible for this study:

- Heather Harbottle
- DAFM staff

The following table presents the objectives and the title of each study.

FY	OBJECTIVE/STUDY TITLE	FTE
09	Continue 08 studies. Test other methods for source tracking. Expand the number of strains/serotypes of animal origin to be typed. Expand the study to examine isolates of other *Salmonella* serotypes and other foodborne zoonotic bacteria. Continue development and testing of a DNA microarray platform	1.0
10	Continue the studies with human isolates and compare the results with isolates from food animals. Continue analysis using differentmicroarray designs	1.0
11	Analyze large numbers of strains from various sources using microarray and other methods to detect genetic differences.	1.0

Investigate Effects on Microbial Flora of Oxytetracycline Fed to Tilapia Held in a Recirculating System

All drugs approved for aquaculture must be reviewed for potental effects on the environment and non-target species. In addtion, there have been concerns about the potential for aquaculture drug use to induce changes in antimicrobial susceptibility in aquatic bacteria, with potential negative impact on human health. Few studies exist which have investigated the effects of antibiotics in aquaculture systems on the fish's bacterial flora and the pattern of antibiotic susceptibility in those bacteria. In addition, there is limited information on the effects of antibiotic use in aquaculture on bacteria located in the water column and sediments.

Tilapia are a very important aquaculture species raised for human consumption. Currently, there are no antimicrobials approved for use in tilapia and there is an effort to expand the current approval for oxytetracycline to other fish species, including tilapia. This study is designed to investigate the effects of oxytetracycline (OTC) medicated feed on bacteria isolated from tilapia housed in a recirculating life-support system.

Specifically, we are examining the effect of repeated OTC treatment (2.5 – 3.75 grams per 100 pounds of fish per day for ten days /kg) on bacteria (*B. cereus*) isloated from the feces and filter water. Organisms will be identified by fatty acid methyl ester (FAME) analysis and genetic relatedness using enzyme pulsed-field gel electrophoresis (PFGE). Antimicrobial susceptibility will be assessed using E-test and microbroth dilution test methods which were developed by CVM scientists. These organisms will be compared to (*B. cereus*) isolated from feeds used in this study.

This study will provide data about antimicrobial susceptibility profiles during and after treatment, that will be useful for developing guidance for industry, which defines protocols that can be used for pre-approval studies discussed in the CVM framework document.

The following personnel are responsible for this study:

- Renate Reimschuessel
- Charles Gieseker
- Eric Evans
- ORAU Fellow

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Complete FAME analysis of all B. cereus strains, complete antimicrobial susceptibilty testing of all isolates using E test and microbroth dilution methods,	0.5
10	Complete final report	0.5

Immunopharmacology

Immunopharmacology focuses on the bi-interaction of drugs used in veterinary medicine and the immune system. The purpose of this program is to determine the effect of infectious diseases, e.g., viral, bacterial, parasitic, on pharmacokinetic parameters typically associated with drug use in domestic animals. An immune response initiates the cascade production of numerous cytokines and other mediators of inflammation. Mediators released in response to infection and disease, especially cytokines, show in rodent models and in man to alter the components responsible for drug disposition, metabolism and elimination. Thus, initiation of an immune response to microbial infections will potentially lead to altered pharmacokinetics and or drug efficacy. Changes such as these could potentially result in either unacceptable violative residues in animal-derived food or drugs which now have altered efficacy or toxicity. Results from this program will aid the mission of the Agency to assure a safe food supply by identifying factors affecting drug safety and efficacy in target animals and human food safety.

Work performed in rodent models and in humans has clearly demonstrated that almost all infections, whether bacterial, viral, parasitic, or fungal, can affect various aspects of drug pharmacokinetics. The basic mechanism for this effect is the appearance of cytokines and other inflammatory mediators produced as a consequence to microbial invasion. The infectious agent itself does not usually affect pharmacokinetic parameters. The production of these host factors influences drug disposition in two distinct manners. The first mechanism involves active suppression of the production of Phase 1 and Phase 2 drug metabolizing enzymes. The second mechanism involves the alteration of various pharmacokinetic parameters distinct from the effect on metabolism, e.g., volume of distribution, clearance. During an infectious disease, either or both mechanisms may be in effect, depending upon the drug. Changes in drug metabolism are a consequence of cytokine action at the gene level, whereas changes in other pharmacokinetic parameters may be due to either temporary changes, e.g., altered blood flow, or permanent changes, e.g., end organ damage.

The net effect of all these changes is either elevated, and possibly violative, drug residue levels, even though FDA approved withholding times were followed, decreased drug efficacy, or increased drug toxicity. However, apart from a few other published studies, minimal work outside OR has been conducted. The effect of disease processes on drug pharmacokinetics in food-producing animals has been determined in this program by using natural and artificial infectious disease models. This work will help to establish infectious disease models for use by regulated industry.

The other aspect of this research program focuses on inadvertent modulation of the immune system as an unintended consequence of drug administration. In addition to strictly drug-induced immunomodulation, the complex interactions of environmental factors, e.g., diet, sanitation, environmental toxicants, together with drugs commonly used in animal husbandry/veterinary medicine, can all serve to modulate the normal homeostasis of the immune system, either alone or in various combinations.

The goals of this aspect of the research program focus on ascertaining the extent to which drugs and xenobiotics will inadvertently alter the normal homeostasis of the immune system in food-producing animals. This effort also necessitates the development of rapid and sensitive methods for assessing immune homeostasis. As a spin off of this aspect of the program, some of these methods can be used to assess immune homeostasis during an ongoing infectious disease for use as an alternative measure of anti-infective agent efficacy.

Determine Effects of Endotoxin and NSAIDs on Inflammatory Cytokines

The FDA/CVM establishes policy for the manufacturing of sterile, pyrogen-free animal pharmaceuticals (injectables) documented in the CVM Policy and Procedures Guidance 1240.4122. The Center has adopted the rabbit pyrogen test (RPT) and limulus amebocyte lysate (LAL) test for monitoring for the presence of pyrogenic substances. The RPT determines a non-specific febrile response to pyrogens, while the LAL test specifically identifies endotoxin (lipopolysaccharide - LPS) and may not detect other potential pyrogens. However, the biological relevance of these tests as it relates to animal exposure is not well understood. There are significant species differences in the magnitude and the type of response to endotoxin, which is further complicated by the dramatic size differences and volumes of pharmaceuticals administered for the treatment of the various domestic species. In light of this, the CVM has assumed a conservative approach to regulating animal drugs based upon the sensitivity of the benchmark testing procedures, and the permissible levels of pyrogen (endotoxin) in manufactured drugs are not specifically mentioned.

The purposes of the current study are to: 1) investigate the physiologic response (primarily body temperature response) of cattle challenged with known low-level concentrations of bacterial endotoxin (*E. coli*), which can then be correlated with responses measured in standard/approved pyrogen testing methods; and 2) identify the lower limit of responsiveness (threshold or "window") to endotoxin in cattle for guidance in the production of sterile, pyrogen-free pharmaceuticals.

Three groups of 8 dairy steers will be challenged with one of three concentraions of *E. coli* endotoxin (1 μg/kg, 0.25 μg/kg, 0.03 μg/kg) and their body temperature and other physiological variables will be monitored and recorded. Calves can be utilized for additional dosing experiments following a 3 to 4 week "wash-out" period, and additional concentrations of endotoxin may be examined in order to more accurately define the threshold for response.

- Jeffrey Ward
- DAR Staff
- Julie Bailey, CVM/ONADE

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Coduct study with 30 stees and three levels of endotoxin; evaluate other endotoxin concentrations, if needed, to find response threshold	1.5

In a separate effort, research is continuing to identify suitable biomarkers for use in clinical trials of anti-pyretic and anti-inflammatory drugs. We have completed the animal phase of a study that used an *E. coli* mastitis model in dairy cattle to identify proteins that were modulated during coliform mastitis. Proteomic techniques including 2D gel electrophoresis followed by MALDI-TOF analyses of tryptic peptides were used to identify proteins that were significantly up-regulated in bovine milk in response to *E. coli*. Follow-up investigations are being conducted to determine the effects of administration of anti-inflammatory drugs on the relative abundance the identified candidate proteins using peptide labeling strategies followed by LC-MS/MS. In addition to analysis via mass spectrometry, a small number of candidate proteins will be evaluated using antibody-based techniques including ELISA to determine if any of the proteins up-regulated in bovine milk during coliform mastitis are suitable markers of inflammation that may be used in evaluating the efficacy of NSAIDs.

The following personnel are responsible for this study:

- Jamie Boehmer
- Jeffrey Ward
- Michael J. Myers
- DAR Staff
- Janis Messenheimer, CVM/ONADE

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Complete the laboratory phase and final report.	1.0

A separate study has been initiated in swine to identify biomarkers of NSAID efficacy, using a combination of biochemical tests, genomic techniques, and flow cytometry. Two separate swine inflammations will be developed that mimic either a systemic or local inflammatory reaction. Using microarray technology, we have identified several genetic markers that exhibit long term changes following in vitro stimulation of porcine blood cells with endotoxin. These markers will be used to determine the impact of Banamine on their expression during an in vivo inflammatory response. In adddition to these genetic markers, we will be evaluating the impact of Banamine on the production of biochemical measures of inflammation such as PGE2, Bradykinin, and Substance P. In collaboration with colleagues at CFSAN, we will also be evaluating changes to white blood cell surface proteins as another functional measure of inflammation and the impact of Banamine on those markers. While most of the research efforts to date have been performed in food animals, efforts will be made to apply the knowledge gained in these studies to companion animals.

The following personnel are responsible for this study:

- Michael J. Myers
- Haile Yancy
- Dottye Farrell
- Sharla Peters, ORAU Fellow
- DAR Staff
- Janis Messenheimer, CVM, ONADE
- Uma Babu, CFSAN

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Conduct study in swine to evaluate impact of NSAIDs on biomarkers of inflammation and pyrexia in swine.	1.0
10	Complete *in vivo* study on impact of NSAIDS during inflammation and pyrexia in swine.	1.5
	Develop protocol to identify biomarkers of inflammation and pyrexia in companion animals that will be used to determine NSAID efficacy	0.2
11	Conduct study to identify biomarkers of inflammation and pyrexia in companion animals that will be used to determine NSAID efficacy	1.3

Metabolism and Residue Depletion

The shortage of drug availability for minor food animal species is well recognized by the Center for Veterinary Medicine. In order to facilitate the approval of drugs in aquatic animals, CVM's Office of Research has begun metabolism studies for identifying a marker residue (MR) potentially present across multiple fish species. Since the metabolism of a compound may vary due to a number of parameters including species, FDA requires radiotracer studies to identify MR in each distinct species for which a sponsor is seeking approval. Before approving a compound for use in food-producing animals, FDA also requires that the sponsor provide an acceptable analytical method capable of reliably measuring the marker residue to ensure that total residue of toxicological concern is not exceeded. We will develop metabolism data and establish a MR for use in support of the development of regulatory analytical methods for veterinary drugs across multiple fish species.

This approach will provide a sounder basis for the choice of an analytical method in a species or a group of fish species which have the same MR. By reducing the need to develop analytical methods for each species, we can speed the drug approval process for various aquaculture species. Additionally, this work should stimulate more sponsors to develop drugs for fish used for human consumption.

Residue depletion studies of known marker residues are also conducted in various fish species in support of validation of regulatory analytical methods.

We routinely coordinate our efforts with those of the National Research Support Project No. 7 (NRSP-7) and participate in their workshops which provide a forum for exchange of ideas among minor species producers, drug manufacturers, researchers, and government agencies on approaches to disease problems and drug priorities.

Residue depletion studies are also conducted in support of guidance development for various manufacturing and human food safety questions.

Determine Marker Residues of Selected Veterinary Drugs in Edible Aquatic Species

In an effort to determine the marker residue (MR) of ivermectin in various finfish species, we will conduct its comparative metabolism and residue depletion studies in various species of fish that are of importance to the U.S. aquaculture. Radiolabelled ivermectin will be used to assist in defining the MR. The literature studies on *in-vitro* drug metabolizing enzyme activities reveal that different fish species vary in their capacity to metabolize drugs.

The metabolism and residue depletion studies will allow us to ascertain fish species with similar or dismiliar metabolic profiles and allow us to identify potentially a common MR of ivermectin in multiple species of fish. Identification of a MR will allow us to develop analytical methods for regulatory monitoring. Additional veterinary drugs, based on the need identified by aquaculture sub-groups, may be studied for finding MR in aquatic finfish species.

Our results on the metabolism and residue depletion study of ^{3}H-ivermectin in rainbow trout, Atlantic salmon, tilapia and channel catfish showed that an unknown metabolite is a potential MR in the muscle tissue of rainbow trout, whereas, parent drug ivermectin is the MR for the other three species. Mass spectrometry and liquid chromatography results indicate that the unknown metabolite may be 3'-O-demethyl-ivermectinB_{1a}. This suggests that rainbow trout metabolizes ivermectin differently than Atlantic salmon, tilapia and channel catfish. In other food animals (cattle and sheep), the parent ivermectin is known to be the MR in their muscle tissue. Metabolism studies of ^{3}H-ivermectin in other fish species, hybrid stried and large mouth are in progress.

The following personnel are responsible for this study:

- Badar Shaikh
- Nathan Rummel
- Renate Reimschuessel
- Charles Gieseker
- Eric Evans

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Animal phase – (a) Complete dosing of ^{3}H-ivermectin to hybrid striped bass (HSB) and large mouth bass (LMB) for full metabolism study; (b) Complete dosing of rainbow trout with non-labeled ivermectin for characterization of the unknown metabolite in muscle. Laboratory phase – (a) Complete HPLC analysis of the incurred ^{3}H-ivermectin in muscle tissues of tilapia, catfish and Atlantic salmon; (b) Initiate Total Radioactive Residue (TRR) and HPLC analysis of the incurred ^{3}H-ivermectin in muscle tissue of HSB; (c) Continue isolation and characterization of a potential marker residue (MR) of ivermectin in rainbow trout muscle using LC & MS.	2
10	Laboratory phase – (a) Complete TRR and HPLC analysis of incurred ^{3}H-ivermectin in the muscle tissue of HSB; (b) Initiate/complete TRR and HPLC analysis of the incurred ^{3}H-ivermectin in the muscle tissues of LMB.	2
11	Laboratory phase – Initiate/complete TRR analysis of ^{3}H-ivermectin in plasma, bile, liver , kidneys and scales of tilapia, rainbow trout, atlantic salmon, catfish, HSM and LMB.	2

Method Development for Improved Drug Residue Testing

Currently, NADA sponsors are required to provide FDA with analytical methods to quantify and confirm the presence of drug residues when a product is approved for use in a food producing animal. These methods are validated through a method trial to establish that the method performs as claimed, that this performance is fit for the intended purpose, and that the technology can be transferred successfully. The methods are the same used to establish the tolerance of the drug.

The approach has served the Agency well; however several issues have developed that need to be addressed. The USDA Food Safety and Inspection Service (FSIS) needs to conduct meat industry surveillance activities using methods that are traceable to the original tolerance. Because technology changes, methods become obsolete. Equipment is no longer available. Some reagents are no longer be used because of environmental and occupational health considerations, Simpler, faster, and safer laboratory techniques are available. Some of the oldest methods, developed before the advent of many modern analytical techniques, are based on total microbiological activity of an extract for which the exact composition is unknown. As drug sponsors develop individual methods for each drug approval, regulatory laboratories have spend scare resources switching between methods including the development of the needed quality control and validation data to demonstrate that the analytical result is valid. Often this causes delays in the analysis of product.

Because the tolerance developed for a drug is directly linked to the method, the relationship between the concentration measured using the original and any new method must be established. The approach taken in the past to develop the needed information has required the direct comparison of analytical results based on the analysis of split samples containing incurred residues, referred to as a bridging study. While this approach is scientifically rigorous, the complexity and resources required for the needed studies has greatly limited the number of bridging studies that have been conducted. The approach also requires bridging studies to be conducted whenever methods are changed.

It is necessary to he initial step in addressing these issue involves developing the information needed to compare the performance of the oldest microbiologically based methods testing total residue of unknown composition with newer chemistry based methods. Because the microbiological activity in the residue may be due to both the drug and metabolites, a linear relationship between a specific marker and the microbiological activity may not exist.

A second goal of the research is to develop data that will support the use of rapid screening tests, such as used in the milk industry, to test meat for drug residues. FSIS would be responsible for implementing new multi-residue quantitative or semi quantitative screening tests at the plant. The use of better screening tests would minimize the number of samples tested by traditional chemical based assays for both determination and confirmation.

Methods in support of NADAs will continue to be evaluated. Typically, the developing laboratory is not involved in the analysis of regulatory samples. The laboratories using the methods usually do not have the same equipment, access to the method developer, or time and resources to modify flawed methods. Usually, the only information available to the scientist using the assay is what is written in the method. Additionally, the scientist developing the method often is an expert in a particular analytical technique; whereas, the scientist using the method is a generalist, and must be able to analyze a wide assortment of samples using a variety of analytical methods. The validation process evaluates the

transferability of a method into a regulatory environment, determines that the methods are suitable for the intended use, and ensures that the written method is clear, complete, and free of ambiguity.

The use of LC-MS/MS methods to quantify drug residues has become widely accepted in recent years due to ease of method development and higher sample throughput. However, these LC-MS/MS methods face some unique problems when transferring to other instruments. The design of each manufacturer's ion source is proprietary and the performance of sources can vary widely in instruments from different manufacturers or even in different models from the same manufacturer. Additionally, the sample matrix present in the extract can significantly change the intensity of the analyte signal. Research is being conducted at OR to investigate matrix effects and develop some tools to help method developers minimize the impact of matrix effects on method transferability. Assessing and controlling matrix effects in LC-MS/MS are significant issues for the entire bioanalytical community, including human drug studies. This research has the potential to not only improve the transferability of methods in CVM method trials but to improve the quality of the data generated throughout the bioanalytical community that FDA depends on for many of its regulatory decisions.

Providing Better Regulatory Methods

OR scientists are working with ONADE, OS&C, and FSIS to design and implement a program to replace obsolete methods with new updated technology. Ideally, multiresidue, multiclass methods base on methods currently used by FSIS will be the basis of the new methods. In FY09, a method will be developed for penicillin G in bovine tissue. While the research will focus on a single analyte, the chromatography and extraction techniques will be designed to allow the incorporation of additional drugs into the method. Knowledge gained in the development of multiresidue methods within the Office of Research will allow the method to be designed to be easily updated with new compounds. The project will serve as the prototype for designing studies to provide updated official methods for use in regulatory purposes. The method will be bridged to the official microbiological penicillin method. The bridging study will require the use of incurred residues.
The following personnel are responsible for these studies:

- Mayda López
- Philip Kijak
- Linda English
- DAR Staff

The following table presents the objectives and the title of the study.

FY	OBJECTIVE/STUDY TITLE	FTE
09	Develop and validate a quantitative and confirmatory method for the analysis of penicillin G and other β-lactams, tetracyclines, macrolides, sulfonamides, and aminoglycosides in bovine kidney.	1.0
	Familiarize and validate the original microbiological method for penicillin G in bovine kidney.	0.5

FY	OBJECTIVE/STUDY TITLE	FTE
	Run a pilot study to gather data to help design the bridging of the new chemical based method to the obsolete microbiological method.	1.0
10	Bridge the new chemical method for penicillin G in bovine kidney to the microbiological method.	1.5
	Begin evaluating the method with additional drugs	1.0
11	Complete the evaluation and documentation of the bridging study and transfer the chemical method to FSIS Labs.	1.0
	Bride additional drugs added to the new method back to the obsolete methods	1.5

Develop Drug Specific Methods

The Office of Research supports MUMS by assisting in method development and validation for needed drug specific methods. In 2001-2004, OR scientists working under a interagency agreement with the USGS, developed a confirmatory method for the marker residue of chloramine-T, p-TSA, in publicly aquacultured fish species. Currently, we have been requested by the Aquaculture Drugs Team in ONADE Division of Therapeutic Drugs for Food Animals to assist in the development of a method for erythromycin in salmon feed. The drug sponsor is developing a research method. OR scientists will modify the research method for regulatory use and validate the method. The project is being coodinated by the USGS Idaho Cooperative Fish and Wildlife Research Unit, University of Idaho.

The following personnel are responsible for this study:

- Philip Kijak
- DRC staff
- Renate Reimschussel

FY	OBJECTIVE/STUDY TITLE	FTE
09	Modify and validate the method for use in the determination of erythromycin in salmon feed	1.0
10	Conduct a method trial of the erythromycin in salmon feed method	0.5

Method Development in Support of Minor Use Minor Species

The need to increase the availability of drugs for minor species has been recognized by the recent passage of The Minor Use and Minor Species Animal Health Act of 2004. By developing methods that can be used "off the shelf" for the quantitation and confirmation of a drug in a minor species, both the Agency and the sponsor will benefit. Multiresidue and multiclass methods enable regulatory agencies to efficiently use resources in enforcing residue tolerances. The need to develop analytical methodology is a major cost incurred by a sponsor submitting a drug for approval which can be minimize or eliminated by the use of an existence of a validated method for the compound of interest or which can easily accommodate the addition of a new compound. By providing tools that both the Agency and drug sponsor can use, the research supports the Agency's Critical Path initiative. Additionally, it can serve as a model of an alternative approach for the development of all regulatory drug residue methods. The current approach to the development of drug residue methods is expensive, sometimes delay the approval of needed drugs, and does not provide efficient methods for the enforcement. The availability of multiclass methodology increases the efficiency of regulatory agencies and enhances their ability to protect the nation's food supply.

Develop Multiresidue Methods

The main emphasis of this program is to develop multiresidue and multiclass methods. Existing multiresidue methods for veterinary drug monitoring are for single classes of drugs, e.g., macrolides, avermectins, tetracyclines. These methods were possible because similar chemical structures enabled a single extraction, purification, and detection scheme. The advent of liquid chromatography/mass spectrometry (LC/MS) instrumentation at prices within the range of routine analytical laboratories changed this situation. MS is a "universal" detector. It is possible to set up conditions that will, within a single chromatographic analysis, detect a wide range of compounds. The high specificity of the MS detector also means that simpler, more "universal," extraction and purification procedures may be used prior to analyzing the extract.

Much of the salmon, shrimp and catfish eaten in the US is a product of aquaculture. Current methods in routine used are capable of monitoring for only a limited number of drugs in aquacultured products using labor intensive procedures. The method developed for the screening and confirmation of mulitple drug residues in shrimp is being evaluated in the ORA Denver laboratory. We have completed developing a screening/confirmation method for finfish such as salmon and catfish. In order to have methods capable of enforcing import tolerances, we plan to extend the multiresidue multispecies methods for shrimp and finfish to include quantitation capability.

We have successfully developed and validated a multiclass, multiresidue method that can quantify and confirm the presence of the following antibiotics in honey: tetracycline, chlortetetracycline, doxycycline, oxytetracycline, ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, sarafloxacin, tylosin, lincomycin, streptomycin, sulfathiazole, fumagillin, and chloramphenicol. Erythromycin and monensin can be detected and confirmed but not quantitated. The method was validated using both fortified and incurred honey. Before the method can be used for regulatory purposes, the validation of the method by an independent second laboratory is needed. New drugs to treat diseases in bees are being tested by the ARS Bee Laboratory in Beltsville and by other laboratories across the US. In addition, recent reports of findings of other non-approved drugs in honey make it imperative that the method be extended to include these additional drugs.

Future research in the development of multiclass multiresidue methods will continue to be in support of the FDA Critical Path Initiative. For example, the multiresidue approach can be applied to drug residue detection in veal calves. As with aquacultured species, multiresidue methods have the

potential to provide both drug sponsors with off the shelf methods to decrease the development costs and the regulatory laboratories with more efficient tools for enforcing tolerances.

The following personnel are responsible for this study:

- Mary Carson
- Philip Kijak
- Hui Li
- Mayda Lopez
- Cristina Nochetto
- Shani Smith

The following table presents the objectives and the title of each study.

FY	OBJECTIVE/STUDY TITLE	FTE
09	Transfer the multiresidue/multiclass method in honey to the Florida Department of Agriculture for second analyst check.	0.25
	Complete development of quantitative multiresidue method in shrimp	1.0
10	Write final report on the development and validation of the multiresidue/ multiclass method in honey.	0.25
	Transfer the multiresidue/multiclass honey method to FDA field laboratories.	0.25
	Transfer the multiresidue/multiclass shrimp quantitative method to FDA field laboratories.	0.25
11	Expand the multiclass/multiresidue honey method to include new drugs that are currently being tested to treat bees and to include other non-approved drugs being misused in beekeeping.	1.0

Method Trials: Chemical

The purpose of the method trial is to establish that the method performs as claimed, that this performance is fit for the intended purpose, and that the technology transfer is successful. Typically, the developing laboratory is not involved in the analysis of regulatory samples. The laboratories using the methods usually do not have the same equipment, access to the method developer, or time and resources to modify flawed methods. Usually, the only information available to the scientist using the assay is what is written in the method. Additionally, the scientist developing the method often is an expert in a particular analytical technique; whereas, the scientist using the method is a generalist, and must be able to analyze a wide assortment of samples using a variety of analytical methods. The validation process evaluates the transferability of a method into a regulatory environment, determines that the methods are suitable for the intended use, and ensures that the written method is clear, complete, and free of ambiguity.

The use of LC-MS/MS methods to quantify drug residues has become widely accepted in recent years due to ease of method development and higher sample throughput. However, these LC-MS/MS methods face some unique problems when transferring to other instruments. The design of each manufacturer's ion source is proprietary and the performance of sources can vary widely in instruments from different manufacturers or even in different models from the same manufacturer. Additionally, the sample matrix present in the extract can significantly change the intensity of the analyte signal. Research is being conducted at OR to investigate matrix effects and develop some tools to help method developers minimize the impact of matrix effects on method transferability. Assessing and controlling matrix effects in LC-MS/MS are significant issues for the entire bioanalytical community, including human drug studies. This research has the potential to not only improve the transferability of methods in CVM method trials but to improve the quality of the data generated throughout the bioanalytical community that FDA depends on for many of its regulatory decisions.

When participating in a trial, the CVM laboratory serves as one of the laboratories conducting the actual hands-on evaluation of the method. The evaluation includes a review of the written method and the analysis of a series of both known and unknown samples

The CVM laboratory plays a crucial role in the sponsor monitored method trial process. Typically, we are the only government laboratory participating in the sponsor's method trial, and, therefore, serve as the only link between the sponsor who developed the method and the end user, the government laboratories. Additionally, the CVM laboratory conducts the only independent evaluation of a sponsor's confirmatory procedure.

Perform Method Trials for the Analysis of New Animal Drugs

Historically, OR participates in one to two method trials for new animal drugs each year. The sponsors of new animal drugs must provide a method for the analysis of the drug if residues at an unsafe level may be present after the approved treatment in the animal. Typically, a regulatory method includes two analytical procedures: 1) the determinative procedure which is used to quantify the drug; 2) the confirmatory procedure which provides a positive identification of the drug.

As part of the validation process for the method, the drug sponsor conducts multi-laboratory method trials of the determinative procedure. One government laboratory, usually OR, participates in these trials. In these trials, the Study Director reviews the method, attends a laboratory demonstration of the method at the sponsor's expert laboratory, and completes a validation of the method as defmed in the sponsor's trial protocol. For a single analyte, the Center's guidelines for method trials typically require a set of thirty samples to be extracted and assayed. Because the data is included as part of the supporting data for the NADA, the studies are done as GLP studies.

OR is the only laboratory that evaluates the confirmatory procedures to determine if they meet CVM performance guidelines. Typically, samples are prepared and extracted by the sponsor and sent to OR for analysis. We will perform analyses at OR.

OR Division of Residue Chemistry Staff are responsible for this study.

The following table presents the objectives and the title of each study.

FY	OBJECTIVE/STUDY TITLE	FTE
09	Start and complete evaluation of sponsors' methods for new animal drugs at CVM laboratory.	0.5
10	Start and complete evaluation of sponsor's method for a new animal drug at CVM laboratory.	0.5
11	Start and complete evaluation of sponsor's method for a new animal drug at CVM laboratory.	0.5

Microbiological Methods

Accurately determining the susceptibility of a bacterium to antimicrobial drugs is of considerable importance to CVM for several reasons. The antibacterial activity of a new antimicrobial compound, submitted by a drug sponsor to the FDA for review, consists of the the drug's *in vitro* activity against the target pathogens. The *in vitro* activity of the drug is the foundation for establishing the dosing regimen and subsequently, the interpretive criteria (*i.e.*, whether a bacterial isolate is susceptible or resistant to the drug). To ensure the accuracy of the susceptibility data, standardized testing methods must be used. Thus, standardized antimicrobial susceptibility testing methods are critical in the antimicrobial drug pre-approval process to ensure that the sponsor used appropriate quality controls. Validated methods are also important, as part assessing the safe use of an antimicrobial in its usage environment, including its effects on other microorganisms. OR scientists are closely involved in validating methods and establishing quality control parameters in association with the Clinical and Laboratory Standards Institute (CLSI). For example, we have conducted studies to develop CLSI standards for antimicrobial susceptibility testing of selected aquaculture pathogens and *Campylobacter*, including quality control organisms and QC ranges for several antimicrobial agents. This type of research is integral to the Agency's mission to approve safe and effective animal drugs.

Develop Antimicrobial Susceptibility Testing Methods and Interpretive Criteria for Aquatic Animal Microflora

In recent years, CVM scientists in our laboratory, in conjunction with CLSI (Clinical Laboratory Standards Institute, formerly NCCLS) developed standardized methods for testing the antimicrobial suscdptibility testing (AST) of aquatic bacteria. There are now two standards: disk diffusion and broth microdilution susceptibility testing methods for group 1 organisms.

It is important to continue this effort since fish pathogens include halophilic Vibrio species (Group 2 organisms) and gliding bacteria (Group 3 organisms). We will start with the gliding bacteria, to optimize growth and assay conditions and will conduct collaborative laboratory trials to test some of these methods.

Susceptibility breakpoints or interpretive criteria for drugs approved for use in food fish, or for any aquatic bacterial pathogen are currently unavailable. Such breakpoints are invaluable for the clinical interpretation of laboratory susceptibility data, providing a predictor of clinical outcome. We have conducted a study to develop interpretive criteria for oxytetracycline, one of the few FDA-approved antimicrobial agents for use in food fish. We are analyzing in vitro susceptibility, pharmacokinetic and pharmacodynamic (PK/PD) parameters, and clinical efficacy during *A. salmonicida* infections in trout.

This project directly supports current and evolving FDA regulatory effors for aquatic and minor species. Data generated from this study will be useful to ONADE by providing reviewers a more thorough understanding of the correlation observed in vivo and in vitro by establishing susceptibility breakpoints. This will help ONADE's efforts to comply with the Animal Drug Approval Act (ADAA) to "facilitate more efficient and expeditious approval of NADAs." In addition, any PK data generated by this study may support efforts to expand drug approvals to other aquatic species.

This project represents a key step toward a research solution to an important problem in aquatic animal health, the lack of CLSI-approved susceptibility breakpoints for any aquaculture bacterial pathogens. This study could serve as a foundation for future efforts to establish interpretive criteria in aquaculture not only in the US, but abroad as well.

The following personnel are responsible for this study:

- Renate Reimschuessel
- Charlie Gieseker
- Eric Evans
- DAR staff
- Ron Miller, CVM/ONADE

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Analyze data from the standard-of-practice or the FDA-approved dosing regime.	0.1
	Conduct bacteriological eradication study to evaluate kill rates and correlate with appropriate in vitro test results.	0.1

FY	Objective/Study Title	FTE
	Analyze the relationship between PK/PD parameters and drug efficacy as outlined in the CLSI guideline M23-A2 (largely from literature) Develop disk diffusion AST methods for gliding aquatic bacteria:	0.1 0.5
10	Conduct multilaboratory trials to test disk diffusion method for gliding aquatic micro organisms. Develop microbroth AST methods.	0.7
11	Conduct multilaboratory trials to test microbroth method for gliding aquatic micro organisms.	0.7

Develop Standardized *In vitro* Antimicrobial Susceptibility Testing Methods for Bacterial Pathogens

We develop *in vitro* antimicrobial susceptibility testing methods to ensure the accurate and reproducible testing of bacterial susceptibility to antimicrobial agents. These methods are performed by exposing a known concentration of a pure bacterial culture to increasing concentrations of a select antimicrobial drug. The endpoint measurement, based on the inhibition of bacterial growth, is reported either qualitatively (susceptible, intermediate, resistant) or quantitatively as the minimal inhibitory concentration (MIC, usually expressed in micrograms per milliliter). To generate reliable and reproducible data, a process of standardization via multi-laboratory studies is required. This effort results in the identification of a suitable quality control (QC) organism and acceptable QC ranges for each antimicrobial agent tested by each testing method, e.g., disk diffusion, broth or agar dilution. OR scientists conduct research in this area in order to improve the quality of antimicrobial data generated for surveillance programs, the drug approval process, to aid clinicians in selecting the appropriate agents, and to provide a means to compare results from different testing laboratories.

The following personnel are responsible for this study:

- Patrick McDermott
- Sonya Bodeis

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Standardization of *in vitro* antimicrobial susceptibility testing. Establish testing conditions for disk diffusion (Kirby Bauer) method for testing members of the genus *Campylobacter*. Evaluate the effects of different components of growth medium.	0.3

Conduct Molecular Characterization of Foodborne Bacterial Pathogens Isolated from Animals and Retail Meats by Microarray Technology

Microarray methods represent the latest advance in molecular technology and offer a discriminatory, highly informative, high-throughput alternative for the parallel detection of hundreds to thousands of genes of interest simultaneously. The application potential spreads across most sectors of the life sciences, including environmental microbiology and microbial ecology; human, veterinary, food and plant diagnostics; water quality control, and industrial microbiology. The technology has been employed successfully for drug discovery, drug evaluation, cancer research, microbial pathogenicity, mechanisms of antimicrobial resistance, as well as genomic "fingerprinting" and detecting genetic polymorphisms of microorganisms.

The objective of this study is to develop a DNA microarray for detecting and characterizing antimicrobial resistance genes and other genes of interest in Gram-negative enteric foodborne pathogens isolated from retail meat samples from the NARMS program as well as from humans, and animals. The genes conferring resistance which correlate to common resistance phenotypes as well as important virulence and toxin genes in *E. coli*, *Salmonella,* and *Campylobacter* species will be consolidated on a single DNA chip or investigated more intensely on individual chips. We will screen for the presence of antimicrobial resistance genes in *E. coli*, *Salmonella*, and *Campylobacter* using DNA microarray and determine the correlation between resistance phenotypes and genotypes.

The following personnel are responsible for this study:

- Heather Harbottle
- Shaohua Zhao
- DAFM Staff

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Complete preliminary studies, optomizing protocols and procedures.	1.5
10	Develop different array designs. Continue studies to optomize protocols investigating antimicrobial resistance genes present, resistant phenotypes, and other virulence traits.	1.5
11	Continue to expand studies to include DNA comparative genomic hybridization studies, as well as expression microarrays investigating resistance traits.	1.5

Pharmacokinetics/Pharmacodynamics

Investigate Antimicrobial Pharmacokinetic Differences Between Normal and Diseased Animals

The target of antibacterial chemotherapy is the bacterial pathogen causing the disease. The concentration of drug required to inhibit the growth of, or kill, the bacterial pathogen is pathogen dependent. In other words, different bacterial strains, often of the same species, may be inhibited by substantially different concentrations of drug. Bacterial resistance to antibacterial agents is also bacteria and antimicrobial agent dependent. While for some bacteria/antimicrobial agent combinations the selection of resistant strains is not an issue, for others resistant pathogens are readily selected when the concentration of the drug nears the concentration required to inhibit the bacterium's growth (MIC). The length of exposure may also be a contributing factor to the selection of resistant bacteria.

The concentration of drug to which the bacterial pathogen is exposed within the site of infection is dependent on the pharmacokinetic behavior of the administered drug in the target animal species. Therefore, it is important to identify those pivotal variables that can affect the pharmacokinetics of the compound in question. In this regard, there is an abundance of information indicating that disease can significantly affect drug activity/availability, distribution, elimination or metabolism. Within the human drug arena, these variables are accounted for by the use of thousands of patients enrolled in clinical trials. However, within the veterinary drug arena, economics necessitate that studies be conducted with a very limited number of subjects (generally no more than 100 per indication). Consequently, it is necessary that CVM use pharmacokinetic/pharmacodynamic (PK/PD) surrogates to ensure that dosage ranges associated with our drug approvals adequately cover the range of situations encountered under actual field use. Unfortunately, essentially all pharmacokinetic data submitted to CVM are generated in normal, healthy animals. Therefore, CVM is unable to validate the assumption that there are unaltered pharmacokinetic parameters in the face of disease for that drug/disease/animal species. Should this assumption be incorrect, we are at risk not only of therapeutic failures, but of equal or greater importance, may also be risking the public health via the inadequate exposure of pathogen to the drug. This in turn, may result in an increased risk of bacterial resistance; therefore, this research is particularly timely given the recently released draft guidance titled "Evaluating the Safety of Antimicrobial New Animal Drugs with Regards to the Microbial Effects of Bacteria on Human Health Concern".

This research will help CVM identify those (if any) classes of compounds where we need to factor disease condition into our human food safety assessments. This information will be invaluable in designing pre-approval studies for assessing the potential for selecting for resistant bacterial strains.

These studies will focus on investigating the differences, or similarities, in the pharmacokinetic behavior of antibacterial agents in normal and diseased animals. The pharmacokinetic studies will investigate dosing regimens bracketed around the optimal dose in order to evaluate the effects of drug concentration on clinical efficacy and selection for resistant pathogens and commensals. It will also determine vascular and extravascular drug concentrations as they relate to the MIC of the pathogen, clinical response and changes in MICs. Each aspect of this investigation will be integrated via PK/PD assessments in an effort to optimize our predictions of the dose (amount, duration, frequency) needed to achieve a specific microbial outcome and the risks associated with failure to achieve the targeted level of pathogen exposure to the drug.

We have completed pilot study of the macrolide class of antimicrobial agents (tilmicosin), initiated because of current issues being presented to ONADE and the absence of information within either the human or veterinary literature with regard to PK/PD criteria to support the selection of an upper

limit to a dosing range. A larger study is underway to examine the effects of disease on tilmicosin pharmacokinetics, as well as to investigate the possible disease effects on the pharmacokinetics of another macrolide (tulathromycin).

The order in which these species will be studied will depend upon resource availability within the Office of Research. In addition, the inclusion of sheep as a fourth species is highly recommended to test our current assumption that substantial evidence of effectiveness for minor species can be based solely upon comparable blood drug concentrations. Enrofloxacin was the first therapeutic moiety to be examined since it is currently approved for use in food producing animals and because of issues raised regarding potential human health ramifications relative to its use in these animal species. We are currently conducting a study of the macrolide class of antimicrobial agents (tilmicosin and tulathromycin) because of current issues being presented to ONADE and the absence of information within either the human or veterinary literature with regard to PK/PD criteria to support the selection of an upper limit to a dosing range.

The following personnel are responsible for this study:

- Jeffrey Ward
- Jamie Boehmer
- DAR Staff
- Marilyn Martinez, CVM/ONADE

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Complete the laboratory and pharmacokinetic data analysis for tilmicosin and tulathromycin. Prepare final report.	0.7

Compare Pharmacokinetics of Small-Ruminant Antiparasitics of Clinical Importance

Selection of dosing regimens for sheep and goats are often based on the assumption of close similarity between these two species (and moreover, their similarity to the major speceis, i.e. cattle). The literature suggests a high degree of similarity among domestic ruminants in the distribution and elimination of drugs that are not metabolized, but eliminated by passive processes, such as renal glomerular filtration. However, it is also known that wide differences in metabolic capabilities often exist among the species that are phylogenetically related.

There is a wide range of available literature concerning pharmacokinetics and drug disposition of antiparasitics in sheep and goats. For some of the commonly used small ruminant antiparasitic drugs, there is no pharmacokinetic information available that would support proposed dose/dosing regimens. For many of these drugs, data is available in sheep and cattle, but totally lacking in goats, even though they are being used in goats. Those drugs that are approved in goats through use of the minor species act have dosing regimens that simply reflect the major species' dose.

These studies will be used to pharmacokinetically characterize small ruminant antiparasitic drugs so that their doses/dosing regimens are based on the pharmacological understanding of the drugs, rather than assumptions of and extrapolations from their similarities to other drugs/species. . To facilitate comparisons, all drugs were tested in all three species, namely, cattle, sheep, and goats.

The following personnel are responsible for this study:

- Joseph Kawalek
- Karyn Howard
- Jeffrey Ward
- Jamie Boehmer
- DAR Staff
- Sanja Modric, CVM/ONADE

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Completion of animal phase and analyses of six antiparasitic drugs in goats, sheep, and cattle. Complete data analysis and prepare final report	1.5

Characterize and Functionally Analyze Bronchial Antimicrobial Peptides Using an Animal Pneumonia Model – A Critical Path Study

Antimicrobial peptides (AMPs) are short proteins that possess direct antimicrobial activity. These compounds have been studied extensively in humans and various animal species, where they are expressed largely at the mucosal surfaces of the intestine and lung. They are a component of the innate immune system and demonstrate antibacterial activity across a broad range of both gram-positive and gram-negative bacteria. Interestingly, members of this group of compounds are also synthesized by certain bacterial species, and two have been used extensively for several decades – ambicin (nisin; a food preservative) and polymixin B (an antibiotic). Two of the largest classes of vertebrate AMPs, and those that have been studied most extensively, are the defensins and the cathelicidins. These peptides are very attractive candidates for development as therapeutic agents because of their selectivity, broad-spectrum activity, and speed of action. Additionally, it is believed that bacteria will not easily be able to develop resistance to them because of their mechanism of action, which is to disrupt the basic structure of the bacterial membrane.

The study of these innate defenses against bacterial infections in the lung may be easily investigated using a bovine pneumonia model in which we have direct access to the airways and their secretions. For the past 5 years we have been performing tracheostomies in beef cattle in order to obtain bronchial fluid for PK/PD analyses of approved antimicrobial agents directed against a common form of bacterial pneumonia. For this study, we used 6 steers that underwent the surgical preparation (tracheostomy), followed by a 7-10 day recovery period. Bronchial fluid was collected for peptide analysis when the cattle were healthy, and again at 6, 12, and 24 hours after the induction of pneumonia (direct instillation of M. haemolytica into the lungs.) Four steers were infected and two served as uninfected (sham) controls. Two-dimensional gel electrophoresis was performed to identify proteins that were stimulated by the infection. Proteins excised from the gels, and/or raw bronchial fluid, were subjected to trypsin digestion and the resulting peptides analyzed by liquid chromatography followed by tandem mass spectrometry (LC-MS/MS). AMPs will be identified by interrogating protein databases with MS/MS sequence data. Antimicrobial peptides of interest will be purified by fractionation using ion-exchange or reverse-phase HPLC.

Once the peptides of interest have been purified, in vitro susceptibility testing will be performed. Minimal inhibitory concentrations (MICs) will be determined against the bacterial strain used for infection (*M. haemolytica*), and for other common respiratory pathogens of ruminant species (*Pasteurella multocida, Haemophilus somnus, Actinomyces pyogenes*.) Standard bacterial susceptibility protocols will be used and will be critically evaluated for their performance with AMPs.

The development of AMPs for clinical applications is already being seen for human therapeutics, and it is anticipated that veterinary applications soon will ensue. In addition to the evaluation of peptide activity, toxicity, and efficacy/clinical performance, the FDA will also need to be prepared to evaluate such basic issues as dosage form and delivery mode, as well the other standard parameters such as MIC/break-point determination. However, in addition to the therapeutic applications of these peptides, they may also have great utility as biomarkers of infection or inflammation, or as surrogates in the evaluation of disease progression or response to treatment with more conventional antimicrobial agents. Subsequent evaluation of these compounds in animal models is anticipated to shed some light on their use in this regard, in both humans and animals.

The following personnel are responsible for this study:

- Jeffrey Ward
- Jamie Boehmer
- DAR Staff

- Patrick McDermott
- DAFM Staff
- Kevin Shefcheck (CFSAN)

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Isolation and purification of AMP(s); *in vitro* susceptiblity testing with bovine respiratory pathogens	1.0

2 COMPLIANCE

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Introduction

The Office of Research supports CVM's compliance activities through research including method development, method trials, test kit validation, and residue depletion/tissue-fluid correlation studies. Reseachers at OR interact with CVM OS&C, CFSAN, ORA, USDA-FSIS and other government agencies in designing and completing research to address compliance issues. How OR accomplishes compliance oriented research is detailed in the following sections.

Method Development

The Method Development program addresses the need for analytical methods of drug residues in animal-derived foods. Some examples of why analytical methods have grown in importance are:

- While drug sponsors are responsible for developing methods for new animal drugs, the Animal Medicinal Drug Use Clarification Act (AMDUCA) allows the extralabel use of animal drugs by veterinarians.
- Import products may contain residues of drugs not allowed for use in the U.S.
- Food monitoring programs cannot effectively rely on single compound methods. FDA and FSIS require regulatory methods that can detect and measure a broad range of drugs at very low concentrations, yet are rugged, fast, economical, and safe. Violative residues of approved drugs or illegal residues of unapproved drugs may indicate improper drug use which could contribute to antibiotic resistance.

Addressing these needs involves incorporating new cleanup, separation, and detection technologies in developing enforcement methods to address agency needs.

Drug Residue Methods

The development of chemical methods of analysis for drug residues in animal products is a major research activity at CVM. Regulatory enforcement methods are used to perform analyses that will support regulatory actions to be taken based on those results. CVM develops regulatory enforcement methods with the intention of transferring these methods to the FDA/ORA field laboratories, USDA Food Safety and Inspection Service, and other agencies with food safety enforcement authority. These methods must be rugged, well defined, validated and meet stringent criteria established for regulatory methods. The high level of performance demanded of these methods is essential, as data generated via these methods must be able to withstand a legal challenge.

In order to perform its mission, it is crucial that the Agency use new tools being developed in the rapidly changing field of chemical analysis. Many techniques, such as electrospray LC/MS/MS, which were considered esoteric or even unknown only a decade ago, are now commonly used on a routine basis in analytical chemistry. New technologies offer the potential to improve methods for detecting and preventing drug residues from entering the nation's food supplies. We must continuously monitor the changes in instrumentation and techniques in analytical chemistry and evaluate new and improved technologies that hold promise for improving the detection of drug residues.

In the summer of 2005, news agencies reported that antiviral drugs were being administered to poultry in China in an effort to control avian influenza. Concerned that the development of resistant viral strains that could be accelerated by agricultural use of these drugs, as well as concerned about unknown direct adverse health effects to consumers of residue-laden meat and eggs, CVM issued an Order of Prohibition banning the use of adamantanes and neuraminidase inhibitors in poultry. Codex Alimentarious has also recommended that regulatory authorities prohibit the use of these drugs in food animals. It is critical that the Agency have available analytical methods to enforce this Order of Prohibition. Developing, validating and implementing such methods is part of the Agency's Pandemic Preparedness Plan. Similar concerns by the Central Science Laboratory, one of CVM/OR's counterparts in the United Kingdom, has led to the development of an informal collaboration between our laboratories.

Develop Needed Enforcement Methods

Multiclass methods are efficient for analyzing a large number of analytes in a single run. However, there are analytes that do not fit into the multiclass method development. For example, certain analytes may require special handling techniques, such as hydrolysis, derivatization, protection from light, or unusual extraction and chromatography. The need for single analyte and single-class multiresidue methods remains high. At OR, the development of such methods is often in response to the request of the Center and the Agency to address regulatory concerns. In recent years, OR has been called upon to develop methods for nitrofuran compounds and antiviral drugs. Custom method development for these prohibited substances incorporates new trends in methodology, data handling, and quality assurance, while meeting appropriate standards for providing valid data. By developing and publishing such methods, OR contributes to the repertoire of all regulatory analysts. Maintaining an active method devlopment program enables OR to fairly evaluate methods developed elsewhere and submitted for review.

The following personnel are responsible for these studies:

- Mary Carson
- Pak-Sin Chu
- Tricia Johnson
- Shani Smith
- Jeffrey Ward
- DAR Animal Care and Use Staff

The following table presents the objectives and the title of each study.

FY	OBJECTIVE/STUDY TITLE	FTE
09	Hormones in animal tissues. Conduct incursion studies and Identify marker metabolites for hormones (continued from FY08)	1.5
	Antiviral Drug Residues in Poulty. Identify metabolites from FY08 incursions. Revise method and validate. Implement surveillance program for antiviral drugs in eggs and poultry. (continued from 08)	2.0
10	Hormones in animal tissues. Validate the method for hormones	1.5
	Antiviral Drug Residues in Poultry. Transfer methodology to other regulatory laboratories. Begin multilaboratory method validation, if needed. (continued from 09)	0.5
11	Antiviral Drug Residues in Poultry. Continue multilaboratory method validation, if needed.	0.5

Method Trials and Validation

The Method Trials program validates analytical methods for drug residues in animal-derived foods and for drugs, additives, and contaminants in animal feeds. Analytical methods for regulatory use are developed by CVM, FDA field laboratories, and new animal drug sponsors. Residue methods test for violative residues of approved drugs or illegal residues of unapproved drugs. In addition to chemical toxicity, residues may contribute to antibiotic resistance. Feed methods test for both potency of medicated products and detection of contaminants.

Methods submitted by other federal or state regulatory laboratories for use as enforcement tools. These methods are validated through the non-NADA method trial program. The goal of the program is to determine if the method performs as claimed, that this performance is fit for the intended purpose, and that the technology transfer is successful. Typically, the developing laboratory is not involved in the analysis of regulatory samples. The laboratories using the methods usually do not have the same equipment, access to the method developer, or time and resources to modify flawed methods. Usually, the only information available to the scientist using the assay is what is written in the method. Additionally, the scientist developing the method often is an expert in a particular analytical technique; whereas, the scientist using the method is a generalist, and must be able to analyze a wide assortment of samples using a variety of analytical methods. The validation process evaluates the transferability of a method into a regulatory environment, determines that the methods are suitable for the intended use, and ensures that the written method is clear, complete, and free of ambiguity.

Our strategy for achieving this goal has several facets: CVM coordinates method trials, participates in method trials, and has a contract with a private laboratory to evaluate methods. Coordination of a trial includes the following:

- Design the trial.
- Find laboratories to participate in the trial.
- Oversee the trial process, provide samples to the participating laboratories, and evaluate the results of the trial.
- When participating in a trial, the CVM laboratory serves as one of the laboratories conducting the actual hands-on evaluation of the method. The evaluation includes a review of the written method and the analysis of a series of both known and unknown samples.

Perform Method Trials to Determine Drug Residues from Unapproved Uses

Animal drugs are used in unapproved ways, so the federal government needs to monitor the nation's food supply for these illegal and unsafe drug residues. Before a method can be used in a monitoring program, it needs to be validated. The validation process shows the method performed as stated by the developer, and addresses technology transfer issues.

The goal of this research program is to have well-characterized, reliable methods available for use by regulatory agencies including FDA, USDA, and state laboratories. CVM will both coordinate and participate in the trials of the method. The trials are conducted at FDA, state and contract laboratories.

The following personnel are responsible for this study:

- Philip Kijak
- OR staff

The following table presents the objectives and the title of each study.

FY	OBJECTIVE/STUDY TITLE	FTE
09	Compete multiple laboratory trial of a method to quantify triphenylmethane dyes in salmon	0.5
10	Coordinate and participate in multiple laboratory trial of a method to quantify a drug to be determined.	0.5
11	Coordinate and participate in multiple laboratory trial of a method to quantify a drug to be determined.	0.5

Pharmacokinetics and Residue Depletion

Regulators and consumer organizations recognize that the current monitoring programs sample only a tiny fraction of meat. This is due in part to the expense and time needed for laboratory analysis. The current approach of in-plant screening tests followed by laboratory repeat analysis, liquid or gas chromatographic quantitative analysis, and mass spectrometric confirmation takes too much time. Our work in the development of multiclass, multiresidue mass spectrometric methods is partly aimed at eliminating the need for the less specific chromatographic procedures. We believe that a system of highly specific and sensitive screening tests at the slaughterhouse combined with a minimal number of mass spectrometric confirmatory procedures at the lab could allow for a much larger portion of the food supply to be tested.

In order to apply rapid screening tests at the slaughter plant, tests must be performed on samples that are easily obtained and tested. The physiological fluids such as blood, urine and saliva suit this requirement very well. Secondly, data must be developed that define the relationship between the drug levels in these fluids and in the edible tissues for which tolerances have been established. Part of this program is designed to develop these data using minimally invasive sampling techniques and small sets of animals.

The Animal Medicinal Drug Use Clarification Act (AMDUCA) allows for certain extralabel uses of veterinary drugs by veterinarians. One of the requirements is that the veterinarian must be able to assure that no residues remain at time of slaughter.

Pharmacokinetic studies are used to define drug and metabolite depletion characteristics of drugs which may be administered in an extralabel manner by the attending veterinarian. Physiologically based pharmacokinetic models can be developed which are capable of estimating the post dosing drug withdrawal times associated with acceptable tissue and fluid drug depletion levels. The physiologically based pharmacokinetic model will provide the guidance required to slaughter animals only after estimated withdrawal times to avoid drug residue contamination in food animals.

Pharmacokinetic drug depletion profiles may be combined and evaluated to provide the background data necessary to develop risk assessment tables for the FARAD (Food Animal Residue Avoidance Database). FARAD maintains the largest database of pharmacokinetic data which describe the time-course of drugs and estimates the depletion of residues from tissues of animals. Extended withdrawal time estimates have demonstrated utility in the prevention of drug residues in food animals.

Use Tissue-Fluid Correlations to Predict Drug Residue Levels in Edible Tissues from Food-Producing Animals

Testing for drug residues in tissues from swine and bovine normally occurs after slaughter. As a result, edible tissues with residues that exceed tolerance are declared adulterated and must be destroyed. The analytical methods used to measure these residues in tissues are time consuming to perform and relatively few of the slaughtered animals are monitored for drug residues. This approach is inefficient and economically costly for both consumers and producers, and does not provide assurance of optimal food safety. The use of rapid, inexpensive preslaughter screening tests, similar to those used for milk, based on detection of drug residues in some easily obtainable biological fluid (saliva, plasma or urine) would enable monitoring of more animals and help ensure greater food safety. Preslaughter testing also allows animals with violative residues to be held back until such time as drug residues deplete to safe levels by normal routes of excretion.

The setting of acceptable drug residue tolerances in tissues from food-producing animals is based, in part, on an evaluation of toxicology data and target animal metabolism studies. This information determines which drug residues (parent and/or metabolites) are a food safety concern and which organ/tissue contains the highest residues. Depletion studies also provide data regarding the elimination of drug residue from edible tissues.

Penicillins are inexpensive, readilly available antibiotics often used and abused in the treatment of swine for many types of infections. Unfortunately, the penicillins possess the potential for the production of an allergic response in the consumer of the animal derived food product. In swine, the accepted tolerance for penicillin in edible food is zero. – therefore the presene of any level of penicillin residue in swine tissue is a violation. Penicillin must be depleted to non-detectable levels in the kidney to be accepted as safe for human consumption. The ability to estimate the non-detectable concentration of penicillin in the kidney may be monitored by the corresponding level in the urine and or plasma.

The objectives of this research study include:

- Providing initial validation for the use of kidney-tissue and urine or plasma fluid correlation data as a tool for determining the safety of edible tissues in penicillin treated swine.
- Increasing the efficient use of animal resources by minimizing the wasteful slaughter of animals with drug residues that exceed tolerance and which must subsequently be destroyed.
- Contributing to an increase in food safety by promoting the development and use of rapid test kits for screening drug residues in a larger sampling of animals.

The following personnel are responsible for this study:

- David Heller
- Alberto Chiesa, CDRH
- OR Staff

The following table presents the objectives and the title of each study.

FY	OBJECTIVE/STUDY TITLE	FTE
09	Complete kidney tissue to biological fluid correlation for penicillin in swine.	0.5

Screening Tests

The Office of Research has a key role in the evaluation of commercial screening kits for milk. The National Conference on Interstate Milk Shipments (NCIMS) has mandated in the Pasteurized Milk Ordance, that all milk must be screened for the presence of beta-lactam drugs using methods approved by the FDA. Working in partnership with the AOAC-Reseach Institute, a protocol for the validation of the kits has been established based on well-defined requirements and testing by both the test kit manufacturer and an independent third party laboratory. In 2008, a new test kit was approved for use, and two previously approved tests were removed from the program by the manufacturer due to concerns raised about the test kit performance.

In 2005, an NADA was approved for the intramammary use of ceftiofur. The labels of all the current kits on the market needed to be updated to reflect changes caused by the new approval. The Office of Research and collaborated with the drug sponsor, Pfizer, the NCIMS, AOAC-RI, and the kit manufacturers to generate the data to need to demonstrate the performace of the kits under the new standard. This effort continues as OR maintians a repository of ceftiofur inccurred milk required for kit validation.

The validation of drug residue kits for milk from other lactating animals such as goats, sheep, and water buffalo has become an issue with the NCIMS in recent years. The industry needs tests for these specialty products, but the manufacturers cannot justify completing a full validation as done with the kits for cow's milk because of the small market. To overcome this problem, a simplified protocol, designed for use only with kits that have already been approved for use in cow's milk, has been developed to allow a kit to be shown acceptable for use with milk from other lactating animals. The State of Vermont completed a study to validate test kits for water buffalo milk, and the State of New York has initiated a study to validate test kits for sheep milk using the simplified protocol.

Research at OR has also had a key role in addressing problems or issues with existing kits. . The OR staff works closely with CFSAN Division of Cooperative Programs and the FDA Milk Steering Committee to address problems as they are itentified. Additionally, OR has utilized its expertise in the validation of milk test kits to the evaluation of screening kits for drug residues in other matrices.

Evaluate Screening Test Performance

An important application of screening tests is to protect human health by monitoring the existence of drug residue contamination in food. A functional screening test kit should not detain uncontaminated food. The kit should also not define food as contaminated due to the presence of some natural biological interference which may cause an entire shipment to be rejected and destroyed. Historically, the primary OR effort has been in support of the milk monitoring program in cooperation with the National Conference on Interstate Milk Shipments. The specific performance criteria for milk screening test kits for antibiotics is established in a protocol developed by CVM in a partnership with the AOAC-Research Institute. The studies in this protocol provide sufficient information to assure that the screening test kits are manufactured to consistent specifications and that these tests respond consistently to well-defined levels of antibiotics in milk.

Ongoing research efforts are concerned with the time and temperature stability of each screening test kit. These studies include the reproducible manufacture of every lot of test kits to continuously meet the same sensitivity criteria throughout the test kit expiration period. Studies will also consider the factors that control the shipping requirements of each test kit.

The following personnel are responsible for this study:

- Philip Kijak
- Robert Condon (Contractor)
- OR staff

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Evaluate the performance of new tests submitted for FDA acceptance.	0.5
10	Evaluate the performance of new tests submitted for FDA acceptance.	0.5
11	Evaluate the performance of new tests submitted for FDA acceptance	0.5

Incursion Services

The development of regulatory methods to monitor the food supply is dependent upon developing and validating detection methods. Part of the process of methods validation is determining if the analyte of interest can be qualitatively and/or quantitatively assessed in incurred tissues. *Incursion* refers to the deliberate dosing of an animal to produce residues in the animal's tissues at time of collection/sacrifice. Incursion studies are necessary because method validation requires testing a new method against tissue samples that "naturally" contain drug residues.

OR does not consider it sufficient to test a method solely with samples that are fortified by drug standard added just before extraction. Drug incursion studies are conducted for scientists at OR and at other U.S. laboratories. Drug dosing may follow a therapeutic regime, but in some cases dosing is calibrated solely to produce a desired residue level in a specific tissue. This target level is strictly a function of the level required to validate a method. Frequently, the Study Director researches the topic to determine how to dose the animal to produce this drug level. Real-time analytical support (as the tissues are collected) may be necessary to determine that the desired level is obtained. Incursion studies are conducted as the need arises and as requests are received. We expect to conduct approximately six studies per year for incurred residues in poultry, eggs, milk, meat, or fish.

We are planning to provide incurred tissues for a number of high priority drugs for aquaculture in the next three years. These tissues will be used to validate residue methods which will be useful for the establishment and enforcement of import tolerances for these drugs.

Some of the incurred residue studies are conducted at the request of ONADE to obtain direct information regarding drugs under review. In addition, some of these studies reflect cross-center collaborations, using the resources at CVM to leverage toward common FDA goals.

The following personnel are responsible for these studies:

- Renate Reimschuessel
- Charles Gieseker
- Eric Evans
- Jeffrey Ward
- Jamie Boehmer
- OR staff

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Incurred Tissue Request: Tricaine 3-aminobenzoic acid ethyl ester in multiple fish species and prepare final report	0.1
	Incurred Tissue Request: multiple drugs for multi-residue method development, prepare final report.	0.2
	Incurred Tissue Requests for Aquaculture Research Taskforce	0.2
	Incurred Residues of Hormones in Bovine Tissues	0.3
10	Incurred Tissue Requests for Aquaculture Research Taskforce	0.3

3 POST-APPROVAL MONITORING

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Introduction

An active research effort focused on survey-oriented research represents an important tool to assist the regulatory mission of FDA. The Survey program is primarily the research-oriented half of an epidemiological approach to the gathering, analysis, and dissemination of data. The sole purpose of a valid, well-designed survey instrument is the collection of information about the status of a particular environment. As such, this program represents a different type of research activity, one in which the pursuit of knowledge does not follow the typical hypothesis-driven research efforts. However, the knowledge base acquired by survey-oriented research is no less important than the results produced by what is considered the traditional form of research; and can be used as a foundation to formulate hypothesis driven studies. This type of research is one for which FDA is well-suited to undertake, especially as the data are often essential for the Agency to fulfill its regulatory mission.

Static and Non-Static Surveys

Survey instruments are subdivided into two groups: static and non-static surveys. *Static* surveys gather information as a "snapshot in time." This survey type is designed to acquire information concerning the status of a given problem or issue at that moment in time. This static assessment is focused on a pre-determined period of time with finite boundaries. If the information collected contains data from properly preserved and reliably archived samples, the information generated allows for a valuable look at past trends also. *Non-static* surveys collect data continually without any pre-determined time limits. This survey type documents changes over time. It is also able to monitor subtle shifts which may lead to activities, such as initiation of intervention strategies and policy guidelines, that can mitigate an identified area of concern.

The databases generated by either type of survey impact the regulatory efforts of CVM and the Agency in several ways. Survey-derived databases can be involved in the following:

- Designing effective monitoring programs to provide real-time assessments. These survey programs are essential in ongoing efforts to predict future trends such that critical decisions can be timed to prevent development of possible adverse consequences.
- Generate risk assessment documents, which provide a quantitative determination of the relative risks that may accrue as a consequence of a given activity. While a risk assessment document does not, by itself, generate new policy, it can lead to the creation of new regulations.
- Directly lead to regulatory decisions through the illumination of critical public health issues.

Current Survey Activities

The principal focus of ongoing microbiological surveys is to understand how antimicrobial agents currently approved for use in veterinary medicine alter the antibiotic susceptibility patterns among zoonotic foodborne pathogens. The targeted bacteria include the pathogens *Salmonell*a and *Campylobacter*, as well as commensal organisms such as *E. coli* and *Enterococcus*. Information to critically assess how antibiotics used in food-producing animals alter bacterial susceptibility patterns is limited. While this data gap is acknowledged by numerous regulatory and academic research entities, the focus of our research activities is uniquely crafted to address critical, mission-critical functions. Current research activities are designed to fill many of these crucial data gaps, so that ongoing efforts to address the role of agriculture and aquaculture on resistance development have a firm basis in science.

OR scientists are responsible for the study design and continued testing of bacterial isolates from the Retail Meat Arm of the National Antimicrobial Resistance Monitoring System (NARMS). The NARMS retail meat program began in January 2002 and has become the third component of NARMS (in addition to the humans component -CDC and the animal component -USDA). The NARMS retail meat group collaborates with the Centers for Disease Control and Prevention to conduct retail meat sampling through 9 FoodNet Sites (California, Colorado, Connecticut, Georgia,Minnesota, New Mexico, New York, Tennessee, and Oregon) plus the Pennsylvania State Health Department. Each site purchases 40 retail meats per month (including 10 each of chicken breasts, pork chops, ground turkey, and ground beef). All ten sites culture the rinsate from each meat sample for the presence of *Salmonella* and *Campylobacter*. In addition, three sites (GA, OR, and TN) culture the rinsates for *E. coli* and *Enterococcus*. Bacterial isolates are sent to CVM's Office of Research where species identification confirmation, antimicrobial susceptibility testing and DNA fingerprinting (PFGE) takes place. As part of this, a database has been developed that provides query capabilities for over 20 specific reports. These reports are provided quarterly to the FoodNet sites and other participants. The FDA NARMS retail meat annual reports are available at http://www.fda.gov/cvm/narms_pg.html. The information published in these reports provide a critical link toward developing needed data in the farm to fork continuum to determine trends in antimicrobial drug susceptibility/resistance among foodborne bacteria and possible associations with antimicrobial drug use in food producing animals.

Another survey research activity is our ongoing participation in CDC's PulseNet program. PulseNet is an epidemiological database focused on the acquisition and storage of DNA fingerprints generated by the procedure known as pulsed-field gel electrophoresis (PFGE). This methodology results in the visualization of a pattern of DNA fragments that is unique to a particular bacterial clone. This database represents a powerful epidemiological tool to conduct trace-back studies during outbreaks of foodborne illness. Our efforts focus on characterizing bacterial strains obtained from animals and retail meats. Data from these samples provide a critical, missing link in the ability of CDC and other state and federal agencies to trace the source of a given isolate back to the farm. Our effort is also unique in two other ways. We are one of a few laboratories that currently submits the antibiotic susceptibility patterns to PulseNet. These data provide a link between two different epidemiological monitoring programs, PulseNet and NARMS. The majority of the samples we are examining for PulseNet are obtained from the NARMS survey. We are also the primary source of *Campylobacter* PFGE patterns for the PulseNet program.

Individual Programs

Participate in PulseNet: DNA Fingerprint Foodborne Pathogens by Pulsed-Field Gel Electrophoresis (PFGE)

In recent years, bacterial subtyping/fingerprinting has became an integral part of epidemiological investigations of foodborne outbreaks. Differentiation of bacterial isolates by DNA fingerprinting has become an essential tool in correctly identifying potential common-source exposures and tracing the source of contamination in food products. In addition, bacterial subtyping has proven useful in understanding how pathogens persist in animal reservoirs. This informatioon has helped to develop specific prevention strategies to control foodborne pathogens at the farm level. To date, the most effective bacterial subtyping method is pulsed-field gel electrophoresis (PFGE).

PulseNet is a national network of public health laboratories that perform PFGE DNA "fingerprinting" on bacteria that may be foodborne. The network permits rapid comparison of these "fingerprinting" patterns through an electronic database at the CDC. PulseNet was established in 1996 through a collaborative effort of CDC, FDA, USDA and state health departments. PulseNet uses PFGE profiles to trace the source of a foodborne illness outbreak. In the past, PulseNet was focused on foodborne pathogens isolated from patients and foods because foodborne pathogen isolates from animals are limited.

In CVM's ongoing surveillance, we subtype *Salmonella* and *Campylobacter* isolated from food animals (cattle, swine, chicken and turkey), retail foods and humans. The isolates are obtained from diagnostic laboratories in the U.S. and NARMS. We will use PFGE to subtype these isolates and compare them to human clinical isolates through PulseNet, and to determine if there is a clonal spread of isolates between animals and humans or widespread dissemination of unrelated strains. We will also determine the antimicrobial susceptibility profiles of these isolates. When antimicrobial resistance is detected we will identify the genetic elements contributing to this resistance. The data generated from this study will help us understand the genetic diversity of *Salmonella* and *Campylobacter* and the types of foodborne pathogens shared between animals and humans. This will allow us to develop better strategies to prevent foodborne pathogens transmitted from animals to humans. The PulseNet network will facilitate rapid detection of clusters of bacterial pathogens by enabling real-time comparison of bacterial DNA fingerprints at the national level. The ability to identify specific strains of bacteria from diverse sources in a short period of time can play a critical role in allowing the detection of agents of bioterrorism, should they appear within the U.S. The study will also help us better understand how antimicrobial usage in veterinary medicine may influence antimicrobial resistance in foodborne pathogens as well as the mechanism of resistance gene transfer in bacterial pathogens of animals and humans.

Thus, the objectives of this project are to coordinate the ongoing DNA fingerprinting laboratory at CVM for subtyping *Salmonella* and *Campylobacter,* to characterize antimicrobial resistance of these isolates, and to participate in the national DNA fingerprinting network for foodborne pathogens (PulseNet).

The long-term goals include:

- Establish and maintain our own DNA fingerprinting database for important foodborne pathogens, including *Salmonella, E. coli* and *Campylobacter*.
- Collaborate with National Antibiotic Resistance Monitoring System (NARMS) in studying the emergence and spread of multiple drug-resistant pathogens.

- Share information via the Internet with other laboratories throughout the country to rapidly determine a source of foodborne infection and mode of disease transmission.
- Compare PFGE to other typing methods such as MLST, and explore highly discriminatory DNA fingerprinting methods such as microarray.
- Provide service to support other research projects at CVM/OR, such as the Biomarker project, and food safety related epideminological studies.

The following personnel are responsible for this study:

- Shaohua Zhao
- Sharon Friedman
- Jason Abbott
- Althea Glenn
- OR staff

The following table presents the objectives and the title of each study.

FY	OBJECTIVE/STUDY TITLE	FTE
09	Generate PFGE data on NARMS isolates of *Samonella* and *Campylobacter*. Analyze and compare DNA fingerprinting profiles of human isolates associated with cases of foodborne illness through PulseNet. To maintain and continue to update the database as well as share and exchange information with other PulseNet labs. Identify antimicrobial resistance genes and investigate mechanisms of multiiple antimicrobial resistance.	4
10	Continue 09 activities	4
11	Continue 10 activities.	4

Characterize Antimicrobial Resistance among Bacteria Isolated from Retail Meats

CVM continues to strive to provide for the safe use of antimicrobials in animals while ensuring that significant human antimicrobial therapies are not compromised. FDA's main safety concern is that use of antimicrobial drugs in food-producing animals may lead to the emergence of bacterial pathogens (disease-causing organisms) that may be harmful to humans and that are resistant to drugs used to treat human illness. To address these concerns, CVM conducts basic and applied research on the prevalence, propagation, and persistence of antimicrobial resistant bacteria in the animal production environment and on foods of animal origin. CVM, in conjunction with CDC and ten public health laboratories, expanded the National Antimicrobial Resistance Monitoring System (NARMS) to include surveillance of retail meats for antimicrobial resistant foodborne bacterial pathogens.

To better understand the contribution of the food supply to antimicrobial resistance among enteric bacteria and to focus efforts to mitigate antimicrobial resistance, CVM initiated two studies to determine the prevalence of antimicrobial resistant bacteria isolated from retail foods of animal origin. The first study began in FY 2001 and involved sampling of retail meat samples (ground turkey, pork chops, ground beef, and chicken breast) obtained from grocery stores throughout the state of Iowa from March, 2001 to July 2002 on a weekly basis. Retail meat samples were tested for the presence of enterococci, *Salmonella*, *Campylobacter*, and extended spectrum beta-lactamase producing Gram-negative bacteria.

The second study, initiated in FY2002, has become the retail meat part of the NARMS surveillance program and involves CVM personnel from the Office of the Center Director and the Division of Animal and Food Microbiology. Currently, ten FoodNet sites participate (California, Colorado, Connecticut, Georgia, Maryland, Minnesota, New Mexico, New York, Tennessee, and Oregon), along with the Pennsylvania Department of Health. Each site purchases 10 packages of chicken breasts, 10 packages of pork chops, 10 packages of ground turkey and 10 packages of ground beef. All ten sites culture each meat sample for the presence of *Salmonella* and *Campylobacter*. In addition, three sites (GA, OR, and TN) currently culture for *E. coli* and *Enterococcus*. All participating laboratories use similar bacterial isolation methods adapted from the FDA's *Bacteriological Analytical Manual*. Isolates from all ten sites are sent to FDA/OR for confirmation of the identification and antimicrobial susceptibility testing. This is an ongoing project as it has become part of the FDA NARMS program.

In addition to monitoring antimicrobial susceptibility patterns, CVM-OR scientists examine resistant isolates for their carriage of resistance determinants. Data on the genetic mechanisms of resistance provides crucial date for assessing the impact of antimicrobial use and spread, and for assessing assiciated public health risks.

Relevance to FDA and Public Health Impact: The overarching goal of antimicrobial resistant research at CVM is to identify and implement methods to reduce microbial hazards associated with antimicrobial drug use in food animals. This includes research focused on improving our understanding of how bacterial antimicrobial resistance develops, disseminates, and persists in the animal production environment when antimicrobial agents are administered under approved conditions. This research supports the FDA in its regulatory decisions pertaining to food safety by providing scientific data on the emergence and spread of resistant bacterial pathogens from the farm production environment to the retail market. In addition, date generated from these studies are a valuable resource for evaluating new animal antimicrobial in accordance with the recommendations of the CVM Guidance for Industry #152. A comprehensive research effort will help ensure that any actions taken to control antimicrobial resistance will be based on sound science. The Agency is devoting resources in these important areas to help fulfill its dual mission to support the safe use of antimicrobial drugs in food animals and to protect public health.

The following personnel are responsible for this study:

- Patrick F. McDermott
- Elvira Hall-Robinson
- DAFM Staff

The following table presents the objectives and the title of each study.

FY	OBJECTIVE/STUDY TITLE	FTE
09-11	Coordinate isolation of *Salmonella, Campylobacter, Enterococcus* and *E. coli* from retail meats from 10 testing sites Confirm identification and determine antimicrobial susceptibilities Determine genetic relatedness of recovered isolates Identify genetic elements causing resistance to antimcrobial agents.	6

Characterize Antimicrobial Resistance among Historical Isolates of Foodborne Pathogens

CVM has contracted with The American Type Culture Collection (ATCC) to acquire banked collections of foodborne pathogens (*Campylobacter, Salmonella, E. coli*) and to test their susceptibility to antimicrobial agents as a means of evaluating the evolution of drug resistance over time. The overall objective of this study is to assist CVM in addressing the public health impact of antimicrobial use in food-producing animals.

ATCC generated susceptibility data on the isolates, and CVM obtained these strains for genetic analyses. Studies are underway to determine the genetic bases of antimicrobial resistance using standard molecular methods. Strains will be screened for known resistance and virulence genes, compared based on plasmid profiles. As needed, additional susceptibility testing may be performed to more thoroughly characterized resistance phenotypes. These data will help understand the development and spread of resistance to human and veterinary drugs over the past 6 decades.

Isolates from animals and humans will be characterized for genetic relatedness in order to determine whether there are specific phenotypic and/or genetic biomarkers (used singly or in combination) that can be used to assess the exchange of bacteria between animals and humans. This will potentially provide information on the emergence of antimicrobial resistance in both animals and humans over the past decades.

The following personnel are responsible for this study:

- Patrick F. McDermott
- Heather Harbottle
- OR Staff

The following table presents the objectives and the title of each study.

FY	OBJECTIVE/STUDY TITLE	FTE
09	Continue molecular studies and strain typing experiments. Characterize plasmid content and mobility among select strains	1
10	Continue molecular studies on resistance determinants.	1
11	Continue molecular studies on resistance determinants.	1

Survey Susceptibility of Foodborne Pathogens from Humans, Food, and Animals in Mexico

Fluoroquinolones are used in food animals to treat serious bacterial infections. This use is a concern due to the possibility that fluoroquinolone-resistant foodborne pathogens may be passed to humans through the food supply. The strength of this linkage is unknown. Thus, monitoring for fluoroquinolone resistance among foodborne bacterial pathogens is a critical surveillance tool in food safety.

In this survey we are analyzing isolates of antimicrobial-resistant *E. coli* and *Salmonella* obtained from humans, food animals and retail meats acquired from the ResistVet surveillance system in Mexico. Susceptibility to a range of antimicrobials, including various fluoroquinolones, will be determined. Molecular methods, e.g., pulsed-field gel electrophoresis, will be used to estimate the relatedness of strains from human and retail meat isolates.

The following personnel are responsible for this study:

- Patrick McDermott
- OR Staff

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Analyze *E. coli* and *Campylobacter* isolates from the ResistVet program.	0.1
10	Continue 2009 studies and begin analysis of *Campylobacter* isolates from the ResistVet program.	
11	Complete 2009 and continue 2010 studies	

Characterize Antimicrobial Resistance Mechanisms of Animal Bacterial Pathogens

During the past decade, bacteria that cause human and animal diseases have developed resistance to many antimicrobials commonly used for treatment of human and animal infections. The development of some resistance by bacterial pathogens seems an inevitable consequence of the clinical use of antimicrobial drugs. It is also believed that excessive use in treating animal diseases, including subthrapeutic applications of antibiotics for treatment and disease prevention have played a significant role in accelerating the emergence of antibiotic-resistant bacteria . Bacteria develop resistance through a number of mechanisms, including enzymatic degradation of the antimicrobial, mutation in the antimicrobial target site, active efflux of the antimicrobial across the cell membrane, decreases in the cell wall permeability to antimicrobials, and the development of alternate metabolic pathways to circumvent antimicrobial interference. The phenomenon of resistance can be a naturally occurring trait or acquired from the environment. The majority of quickly emerging resistance phenotypes have acquired extra-chromosomal genes that may cause resistance to an entire class of antimicrobial agents. In recent years, many of these resistance genes have been associated with transferable, extra-chromosomal DNA plasmids, and other mobile DNA elements, such as transposons and integrons. These mobile DNA elements which carry antimicrobial resistance genes can transmit among bacteria via conjugation or transformation. Although much scientific information is available on this subject, many aspects of the development of antimicrobial resistance still remain unclear. The increasing incidence of antimicrobial resistant bacterial pathogens has severe implications for the future treatment and prevention of infectious diseases in both humans and animals. In order to control the emergence and spread of antibiotic resistance, to protect the potency of the currently available agents, understanding the complex ecological and molecular aspects of antimicrobial resistance mechanisms is greatly needed.

The objectives of this project are to identify antimicrobial resistant animal pathogens, characterize genes associated with their antimicrobial resistance and determine how resistance gene transfer occurs among animal bacterial pathogens. The results of this study will allow us not only to reveal mechanisms of antimicrobial resistance, but also facilitate molecular epidemiology investigations of resistance dissemination in bacteria among animals and their environment.

The following personnel are responsible for this study:

- Shaohua Zhao
- Sherry Ayers
- Sonya Bodeis-Jones
- Karen Blickenstaff
- DAFM Staff

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09-11	Identify antimicrobial resistant animal pathogens, characterize genes associated with their antimicrobial resistance and determine how resistance gene transfer occurs among animal bacterial pathogens.	1

Molecular Serotyping of *Salmonella spp*. by using the Luminex multianalyte profiling (xMAP) technology

Serotyping of Salmonellae is a valuable phenotypic subtyping tool for understanding the epidemiology of this important foodborn pathogen. Traditional serotyping is using agglutination test according to the Kauffmann-White scheme based on their O, H, and Vi antigens. Such tests are tedious and have problems associated with antibody production and availability. Luminex multianalyte profiling (xMAP) has provided an alternative method for *Salmonella* serotyping. This technology combines PCR with a multiplexed bead-based suspension array detection system which allows us to detect specific nucleic acid sequences that encode different O and H antigens. The assay has been developed by Center for Diseases Control (CDC). We will use *Salmonella* isolated from the NARMS's retail meats for this study. The results will allow us to determine the best method for serotyping *Salmonella* based on ease of testing, cost, reproducibility, and time efficiency. The project will also support and assist CDC & FDA in the development of molecular serotyping of *Salmonella* using Luminex technology.

The following personnel are responsible for this study:

- Shaohua Zhao
- Althea Glenn
- Sherry Ayers
- Karen Blickenstaff
- DAFM Staff

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09-11	Molecular Serotyping of *Salmonella spp*. isolated from NARMS Retail Meat by using the Luminex multianalyte profiling (xMAP) technology	1

Determine the prevalence of pathogenic *Escherichia coli* recovered from NARMS Retail Meats

Escherichia coli are commonly found in humans and animals. Most *E. coli* are harmless but some are pathogenic and can cause enteric or extraintestinal infections in humans. Human *E. coli* enteritis is often transmitted through consumption of contaminated food, especially products derived from animals, or water. Shiga-toxin producing *E coli* (STEC) and enterotoxigenic *E coli* (ETEC) are most important groups of foodborne pathogenic *E. coli.* In addition, some studies also suggested that foods might serve as a potential source of urinary tract infections caused by uropathogenic E. coli in humans. There is a paucity on information of the actual prevalence of pathogenic *E coli* in retail meats, which is in part due to the lack of routine screening of these pathogens in foods. The objective of the study is to screen for ETEC, STEC and UPEC among *E coli* isolates recovered from retail meat samples collected for NARMS, and to determine the prevalence of these pathogens, and to assess the potential risk of foodborne *E. coli* infections.

The following personnel are responsible for this study:

- Patrick McDermott
- Shaohua Zhao
- Xiaodong Xia, University of Maryland
- Jianghong Meng, University of Maryland
- DAFM Staff

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09-11	To screen for ETEC, STEC and UPEC among *E coli* isolates recovered from retail meat samples collected for NARMS, and to determine the prevalence of these pathogens, and to assess the potential risk of foodborne *E. coli* infections.	1.5

4 ANIMAL FEED SAFETY

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Introduction

Assurance of the safety of animal feed and feed commodities is one of the central regulatory obligations of the Center for Veterinary Medicine. Even though the animal feed industry in the United States is largely self-regulated, there are a number of federal and state standards set for feed manufacturing. These regulations include medicated feed Good Manufacturing Procedures (GMPs) to control potentially unsafe chemical residues in edible animal tissues, and the bovine spongiform encephalopathy (BSE) feed ban to prevent spread of BSE and the potential for variant CJD in humans. The Food and Drug Administration has also promulgated regulations covering low-acid canned pet food and salmonellae contamination of feeds, programs intended to provide some control for dissemination of animal and human food-borne pathogens. Nearly all states have some type of regulatory program aimed at ensuring that feeds sold within their borders are properly labeled to ensure correct and safe usage. Enforcement of regulations of feed and feed commodities in the United States is primarily controlled by state feed control officials in consultation with the FDA.

Within the past year several new issues have arisen in the area of animal feed contamination. First, the intentional contamination of feed and feed ingredients by melamine and cyanuric acid, to artificially increase the apparent protein content, led to widespread illness and death of companion animals. Second, the production of ethanol by fermentation of grain has led to increasing incorporation of the by-products (distillers grain, DG) into animal feed. Antimicrobial processing agents are used to prevent unwanted bacterial growth during fermentation, and analytical methods are needed to investigate whether antimicrobial residues occur in DG or feed forumulated with DG. Also, there is some concern that aflatoxins present in the grain could be concentrated in DG following the ethanol fermention step, so a method development study for mycotoxin residues in DG has also been inititated. Method development in all these areas will continue, with a particular emphasis on extracting the widest range of nitrogen dopant compounds, antimicrobial compounds, and mycotoxins, followed by multiresidue analysis by liquid chromatography tandem mass spectrometry. It is anticipated that all these methods will be transferred to field laboratories for regulatory analyses. Also, a new technology, Quadrupole-time-of-flight tandem mass spectrometry, will be tested for its capability to detect residues with a priori assumptions of what might be present.

The Animal Feed Safety Research Program at the Office of Research is designed to support the regulatory efforts of FDA/CVM. This program is currently oriented in two areas: (1) Development and evaluation of analytical methods for detection of unsafe contaminants in feeds and (2) The conduct of microbiological surveys of feed commodities for contamination with food-borne pathogens. A method allowing detection of bovine-derived rendered materials in complete animal feeds has been developed to provide support for the enforcement of the FDA BSE feed ban.

Recently, there has been some concern expressed about the potential for feed to serve as a vector for the dissemination and maintenance of antibiotic resistant bacteria in animal production environments. Surveys, both internal and extramural have been developed and are underway to assess the role various feed commodities might play in this phenomenon. Several reports of results of this work have been published in the past two years. The intramural survey efforts are continuing.

With the recent development of interest in bio-security of agri-business, especially animal agriculture, there has been a renewed interest in developing and evaluating rapid microbiological screening methods for use in animal feeds and feed commodities. A new project is planned for development in the animal feed safety research area which will begin to gather information about and evaluate rapid screening methods for their applicability to feed and feed commodities in the U.S.

Individual Programs

Use PCR and Immunochemical-Based Methods to Detect Natural and Rendered Materials that May Potentially be Used in Animal Feedstuffs

Currently, the laboratories of FDA's Office of Regulatory Affairs are using a validated PCR-based method to detect rendered bovine derived materials in animal feed and feed ingredients. This method is being used as the confirmatory method for feed samples found postive for mammalian proteins by feed microscopy. The method has a bovine-specific primer set, a porcine-specific primer set, and a multi-species specific primer set that detects the rendered remains of sheep, goats, pigs, cows, deer, elk and horse. As pure horse or pure pig proteins are exempt from the current feed ban, restriction fragment-length polymorphic analysis can determine if the PCR amplicon derived from the multi-species primer set is due to the presence of one of these two exempt materials.

As a consequence of discussions with ORA analysts using this method, we developed a simpler DNA extraction method and a faster PCR method for detection of mammalian materials. The DNA extraction method again uses commercially available reagents that will be marketed as a single DNA extraction kit.. The new PCR portion of this method uses a real-time platform to examine for mammalian mtDNA. A real-time PCR platform also eliminates the post-PCR processing typically needed for traditional PCR, which was the most common source of problems during the validation trial of the PCR-based method currently used by FDA's field laboratories.

We developed specific primers that permit detection of materials derived from either cattle, deer, elk, pigs, sheep, goats, or horse. Results from our extensive in-house evaluation of this method demonstrated that it is capable of detecting those materials down to a concentration of at least 0.1%. This method was evaluated against the same acceptance criteria used in our evaluation of the commercial test kits. The real-time PCR method can also detect bovine, porcine or ovine materials produced in the European Union. Current guidelines for preparation of meat and bone meal in countries of the European Union require conditions of elevated temperature (~133 C) and pressure (2.7 bar). These are processing conditions that are more stringent than those practiced in North America. This method was successfully used in a second analyst check to identify materials derived from either cattle, sheep, deer, or goat. The real-time PCR method represents a significant improvement over the current method, as a single analyst can analyze 12 samples in a little more than 2 hrs. The current method requires 8 hours to analyze these same 12 samples. We have initiated a peer-verification of this method to ensure that other laboratories can perform this method. We anticipate a successful verification of this method in early FY09, at which time we will work with OSC and ORA to migrate this method to our field laboratories.

We have been able to modify the real-time PCR portion of the current method into a multi-plex real-time PCR method. This approach uses a Taqman-style approach to permit detection of five different ruminant species in a single reaction tube; cattle, sheep, goat, deer and elk. The multiplex approach combines the primers for three different species into a single PCR reaction tube, with detection accomplished using a fluorescently labelled probe specific for each species. This test will also permit detection of ruminant proteins rendered in the E.U. The goal of this effort is to create an assay in which both the DNA extraction and PCR assay reagents are contained in two commercially available kits. Validation of this method is contingent upon the staff scientist to be hired using GAP funds.

A second line of research focuses on developing an immunochemical test to distinguish between prohibited and exempt bovine materials. Using a unique approach, we have been able to generate antiseras to two distinct portions of a bovine muscle protein. These antiseras are being use to create a sandwich ELISA, which should yield an assay with a greater senstivitiy than with either antisera alone. This test will permit ta laboratory to ascertain if their PCR positive result for bovine materials

was due to the presence of bovine muscle (prohibited material; positive ELISA result) or some other bovine protein (exempt bovine materials; negative ELISA test result). The long range goal will be to produce monoclonal antibodies of the same specificity. Monoclonal antibodies are a more stable immunological reagent that does not change characteristics over time, like antisera will.

A third area of research would focus on the development of a DNA microarray capable of detecting a large variety of animal species (FPP 2.1.2.1.E). While the initial application would be in the area of feeds analysis for enforcement of the 1997 Feed Ban, this approach can eventually be used for a multiple purposes such as species confirmation to determine accuracy of labelling, identification of filth contamination in feed and food, and identification of microbial contamination of feed.
The following personnel are responsible for this study:

- Michael J. Myers
- Dottye Farrell
- Haile Yancy
- Christine Deaver, ORAU Fellow
- Staff Scientist

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Finalize peer verification of real-time PCR assay. Work with OSC and ORA to transfer method to FDA field laboratories	0.7
	Continue efforts to use the polyclonal antisera to bovine muscle protein(s) in the development of a sandwich ELISA.	0.5
	Begin validation of multiplex real-time PCR methods (tentative.	0.3
10	Begin development of monoclonal antibodies. Continue development of an ELISA for bovine muscle proteins, using a combination of antisera and monoclonal antibodies.	0.3
	Begin validation of ELISA	0.3
	Finalize validation of multi-plex real-time PCR (tent). Begin transfer to field laboratories	0.2
	Begin development of a DNA microarray for detection of animal remains in feed and feed ingredients	0.2
11	Continue validation of ELISA	0.3

Evaluate Commercial Test Kits for Detecting Rendered Materials that May Potentially be Used in Animal Feedstuffs

Unlike human medical devices, veterinary devices for products and drugs regulated by CVM do not require pre-market approval. Thus these devices do not receive regulatory scrutiny unless issues with performance are noted and brought to the attention of the Center. Thus, devices such as Neogen's Reveal for Ruminant Test, Strategic Diagnostics Inc (SDI) Feedchek test, ELISA-Technologies MELISA-Tek test, and Tepnel's BioKit can be offered for sale without having to meet any requirements set by FDA. All four tests are designed to detect one or more different species of processed animal proteins. The Neogen, Tepnel, and ELISA technology tests are marketed as ruminant specific, using the exact same antibodies for bovine muscle, where as the SDI test detects rendered bovine (ruminant), porcine, and avian meals, and is more of a bone-specific test. The SDI and Neogen tests use lateral flow strips, while the ELISA-Technologies and Tepnel's test are a standard 96-well ELISA format assay.

As a direct consequence of the single cow diagnosed as BSE-positive in December of 2003, the Center began an evaluation of the commercially available test kits. As part of this effort, CVM's Office of Research developed a set of acceptance criteria by which to assess the performance of these and similar tests. These criteria were developed were criteria for kits that might be suitable for regulatory use. We found that they could not attain the criteria developed by FDA. The results of our evaluations have been published.

At least one kit manufacturer has purported to have made improvements in the design of their kit to address some of the deficiencies identified in our evaluation. A second round of kit evaluations is therefore desired. However, due to the effort needed to evaluate these kits, this work can not be initiated with current resources. This effort will be initiated if additional resources become available.

The following personnel are responsible for this study:

- Michael J. Myers
- Haile Yancy
- Dottye Farrell
- Christine Deaver, ORAU Fellow
- Yolanda Jones
- Staff Scientist

The following table presents the objectives and the title of each study.

FY	Objective/Study Title	FTE
09	Re-evaluation of the Neogen Reveal for Ruminant test and other tests identified by the manufacturers as modified to improve the test performance.	0.3

Survey Microbiology of Animal Feed and Feed Commodities

The animal feed industry is a parallel industry to the animal production industry. The feed industry manufactures over 100 million tons of feed for animal production, not including that required for dairy herds and for the maintenance of breeding stock.

With the exception of the cereal grains and grasses, virtually all other commodities that comprise animal feeds are by-products of other industries including the animal production industry. The feed industry can be viewed as a recycling business that provides sources of nutrients of high biological value for animal production and for companion animals. It is this process of recycling that may serve as a mechanism for the introduction and maintainence of bacterial pathogens and antibiotic resistance determinants in the animal production environment.

There are many reports over the years demonstrating the role that animal and insect vectors play in the transmission of bacteria in animal production units. There is limited information on the role of feed in the transmission of bacterial pathogens, including antibiotic resistant strains. Most of the information that exists is fragmentary and is targeted at human pathogens, such as *Salmonella* and recently *E. coli* O157:H7. Recent reports of the isolation of synercid-resistant enterococci and methicillin resistant staphylococci from this environment suggest the need for a better understanding of the following:

- Microbial dynamics of feed
- Role feed may play in pathogen transmission
- Establishment and dissemination of antibiotic resistance in the animal production environment

The purpose of this research is to establish a survey program that routinely screens feedstuffs and complete feeds received at OR and those provided by outside collaborators. We establish cultural methods to determine the presence of various organisms of interest, including *Salmonella*, *E. coli*, *Enterococcus* spp., *Staphylococcus* spp., and *Clostridium*. In addition, we will assess the susceptibility of the isolates from these studies to antibiotics used in food-producing animals and in human medicine.

Development of this program and its conduct over time will allow a better assessment of the role animal feed plays in the introduction of pathogens into the animal production environment. The program will also assess the potential for feed to disseminate resistance determinants in the animal population. This program provides the capacity to isolate specific bacteria from feed and feed commodities, which the Division of Animal and Food Microbiology can use in other research studies.

The following personnel are responsible for this study:

- Patrick McDermott
- Peggy Carter
- Joseph Paige, OS&C

The following table presents the objectives and the title of each study.

FY	OBJECTIVE/STUDY TITLE	FTE
09-11	Continue sample analysis on feed samples collected within FDA's Feed Contaminants Program (CPG 7371.003).	1.0

Develop Feed Security Tests

The Office of Research continues to develop feed surveillance methods to enhance feed security preparedness. This project has branched in new directions in response to recent events and concerns.

First, distiller's grain (DG) is a major co-product of dry-grind ethanol distillation. It is rich in nutrients and, as such, is an excellent feed supplement for livestock. However, there is concern that residues may occur in DGs from the antibiotics used to prevent bacterial growth during ethanol fermentation, especially if DGs are subsequently incorporated into animal feed. Surveillance and enforcement methods are needed to support any remedial action to be taken against the incidence of these residues in DG. A multi-class, multi-residue SPE ion trap-LC/MS/MS method was developed to monitor for antibiotics in distillers dry grain. Thirteen compounds have been included in the current phase of this project,. The next phases include (1) extending the method to distiller's wet grain and DG solubles, (2) adding additional target compounds, (3) transfering the method to a triple quadrupole instrument (4) including internal standards for improved quantitation (5) assisting the implementation of the method in field laboratories. Another aspect of this project will be to evalaute the performance benefits of using OR's new quadrupole time-of-flight instrument with this method.

Second, a parallel method development project will continue, with the aim of identifying whether mycotoxins from moldy corn survive the fermentation process and remain in distillers grain by-products. A multi-residue method will be developed to incorporate as many compounds as possible, focussing on aflatoxins. Initial stages have been carried out, including optimization of chromatography and mass spectrometry for detection of standards, and testing of published extractions methods.

Third, the discovery of melamine and cyanuric acid as fraudulent nitrogen dopants in animal feed ingredients has become a significant health and safety question. OR scientists developed and validated an LC/MS/MS screening method for these compounds in a variety of finished animal feeds and feed ingredients. This method will be used to test feeds used in OR research projects, and will be modified to test other nitrogen-rich compounds that might be considered alternatives to melamine or cyanuric acid.

CVM will continue to apply liquid chromatography-tandem mass spectrometry (LC-MS/MS) to new surveillance challenges. As new and existing methods are developed or adapted for the new target compounds and matrices, the provisional methods will be subjected to second analyst checks and method validation. Meanwhile the on-going effort to develop LC-MS/MS methods for possible chemical contaminants and toxins in feed will continue. This aspect of the study has now been underway for three years and although significant progress has been made, much remains to be done. CVM will seek to apply rapid screening and LC-MS/MS to the surveillance of a variety of animal feeds for deliberate contamination by drugs, pesticides, and/or other toxins.

CVM's role in a food security crises is to provide the scientific support through the development of rapid and specific agent detection capabilities. The rapid detection of agents, or worse, the validation of rumors of agents, is a critical requirement for decisions regarding the magnitude of the crisis and solutions to the problems. A rapid assessment of the presence or absence of broad classes of biological agents or chemicals may be acquired through the use of commercially available rapid screening kits. Quantitative and/or qualitative determination of the specific biological or chemcal agents are often accomplished with state-of-the-art LC-MS/MS analytical procedures. Aflatoxin is a naturally occurring mycotoxin which may contaminate feed ingredients or may be added intentionally to adulterate prepared feeds. The sensitivity and specificity of two commercially available, AOAC approved aflatoxin screening test kits (Agri-Screen by Neogen and Afla Test P by Vicam) are being evaluated in corn contaminated with known levels of naturally occurring aflatoxin. The same test systems are also being applied to corn samples to which feed components have been added and to the final animal feeds into which the aflatoxin contaminated corn has been

incorporated. The same performance evaluation in feed ingredients concept followed by validation in final feed products may be applied to additional commercially available screening test kits for other naturally occurring mycotoxins.

Some unknown threat agents may involve security issues which will limit the ability of CVM to study these agents, however, in some cases sufficient information may be gained through the use of "simulants" and the principles may also be applied to similar unknown agents. Finally, a new intelligence-gathering scheme is planned to enhance CVM's ability to identify potential threat agents to include in method development.

The following personnel are responsible for this study:

- David N. Heller
- Cristina Nochetto
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FY	OBJECTIVE/STUDY TITLE	FTE
09	Apply the validated LC-MS/MS method for veterinary antibiotic residues in Distillers Grain to the analysis of survey samples. Add additional compounds as needed and transfer the method to a triple quadrupole instrument. Apply LC-MS/MS methods for melamine and cyanuric aicd in multiple animal feed matrices to testing of OR feeds. Add other possible target analytes. Develop and validate multiresidue LC-MS/MS method for fungal mycotoxins in prepared feeds Develop and validate extraction methods for aminoglycosides in feed. Design a novel scheme for intelligence-gathering on potential contaminants in animal feed Validate rapid screening test systems for aflatoxins in feeds prepared from grains	3.0
10	Evaluate LC-Q-TOF for screening animal feed for organic contaminants, without prior assumptions of potential targets. Continue laboratory analyses as needed, data compliation and write-ups of method procedures . Complete method validation and testing of various provisional methods, prepare method standard operating procedures and reports. Identify and evaluate additional rapid screening test systems for toxins in grains and prepared feeds	1.5
11	Transfer methods to field laboratories for second analyst checks, multi-laboratory trials and application in feed surveillance	1.5