V(A). Planned Program (Summary)

Program # 6

1. Name of the Planned Program

Diseases of Animals as Important Inhibitors of Food Security

☑ Reporting on this Program

V(B). Program Knowledge Area(s)

1. Program Knowledge Areas and Percentage

KA Code	Knowledge Area	%1862 Extension	%1890 Extension	%1862 Research	%1890 Research
121	Management of Range Resources			5%	
132	Weather and Climate			5%	
135	Aquatic and Terrestrial Wildlife			10%	
303	Genetic Improvement of Animals			10%	
311	Animal Diseases			20%	
312	External Parasites and Pests of Animals			10%	
313	Internal Parasites in Animals			10%	
314	Toxic Chemicals, Poisonous Plants, Naturally Occurring Toxins, and Other Hazards Affecting Animals			10%	
704	Nutrition and Hunger in the Population			10%	
712	Protect Food from Contamination by Pathogenic Microorganisms, Parasites, and Naturally Occurring Toxins			10%	
	Total			100%	

V(C). Planned Program (Inputs)

1. Actual amount of FTE/SYs expended this Program

Year: 2013	Extension		Research	
	1862	1890	1862	1890
Actual Paid Professional	0.0	0.0	11.6	0.0
Actual Volunteer	0.0	0.0	0.0	0.0

2. Actual dollars expended in this Program (includes Carryover Funds from previous years)

Extension		Research	
Smith-Lever 3b & 3c	**1890 Extension**	**Hatch**	**Evans-Allen**
0	0	247293	0
1862 Matching	**1890 Matching**	**1862 Matching**	**1890 Matching**
0	0	1355174	0
1862 All Other	**1890 All Other**	**1862 All Other**	**1890 All Other**
0	0	6275966	0

V(D). Planned Program (Activity)

1. Brief description of the Activity

Global food security represents access to food at many levels. Worldwide, millions of people live hungry due to poverty or lack food security intermittently. As theglobal population grows, the need to develop better ways of providing enhanced food quality and production and improving land use whilepreventing food degradation, as well as distributing the food efficiently and without spoiling, will become key research foci.The health of the animal food sources, as well as plants ources, is important to maintain and expand the nutrition of populations. However, disease is still common in food sources. Many pathogens evolved to survive in the prevailing conditions existing during thecourse of food production and food conservation may be deficient even in the developed world. Further, if the source of the food is diseased, for example, Johne's disease in cattle or Vibrio tubiashii in seafood or Clostridium perfringens infections in several mea tanimals (pork, poultry, etc), the security of food will be compromised. This project will address aspects associated with food animal security, that is, developing diagnostic tests and vaccines and creating a better understanding of the mechanisms of pathogenesis of or immunity to many virulent bacteria, viruses and health conditions.

2. Brief description of the target audience

Animal disease professionals
Animal producers
Food processors
Shellfish industry including growers and hatchery operators
Pharmaceutical companies
World health organizations
FDA
USDA APHIS
Policy Makers
CDC

3. How was eXtension used?

eXtension was not used in this program

V(E). Planned Program (Outputs)

1. Standard output measures

2013	Direct Contacts Adults	Indirect Contacts Adults	Direct Contacts Youth	Indirect Contacts Youth
Actual	25	70	210	0

2. Number of Patent Applications Submitted (Standard Research Output)

Patent Applications Submitted

Year: 2013

Actual: 0

Patents listed

3. Publications (Standard General Output Measure)

Number of Peer Reviewed Publications

2013	Extension	Research	Total
Actual	0	6	0

V(F). State Defined Outputs

Output Target

Output #1

Output Measure

- 1. To study mechanisms of important bacterial diseases affecting food sources in seafood production and meat production.

Year	Actual
2013	0

Output #2

Output Measure

- 2. To create diagnostic approaches to important conditions such as bovine herpesviru

Year	Actual
2013	0

Output #3

Output Measure

- 3. To develop strategies to increase immunity, including the development of vaccines, against pathogens that impact the food sources.

Year	Actual
2013	0

Output #4

Output Measure

- 4. To study toxins that affect food sources

Year	Actual
2013	0

Output #5

Output Measure

- 5. To enhance partnerships with industry and stakeholders for the application of resilient technologies, practices, and management strategies.

Year	Actual
2013	0

V(G). State Defined Outcomes

V. State Defined Outcomes Table of Content

O. No.	OUTCOME NAME
1	Study mechanisms of important bacterial diseases affecting food sources in seafood production by enhancing the capacity and sustainability of salmon and trout populations.
2	Develop targeted intervention strategies to prevent pathogen contamination in bivalve rearing systems
3	To study mechanisms of important bacterial diseases affecting food sources in meat production.
4	To create diagnostic approaches to characterize the genetic difference between bovine herpesvirus type 1 variants and vaccine strains.
5	Develop strategies to increase immunity, including the development of vaccines, against pathogens that impact food sources. Identify the role of mother cow immunization on calf protection agains MAP.
6	Strategies to increase immunity in animals through dietary supplementation of selenium.
7	Strategies to increase immunity in animals through development of vaccines against influenza.
8	To evaluate the toxicity of various mycotoxins in food.

Outcome #1

1. Outcome Measures

Study mechanisms of important bacterial diseases affecting food sources in seafood production by enhancing the capacity and sustainability of salmon and trout populations.

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual
2013	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
We plan a long-term commitment to understanding Renibacterium salmoninarum pathogenesis, which causes a progressive, multifocal granulomatous disease, Bacterial Kidney Disease (BKD), that involves the kidney and other internal organs.
We have completed the genome sequence of the pathogen and a logical next step is to examine the level of expression of candidate antigens during growth of the pathogen in fish.

What has been done
Collection of RNA was extremely challenging and efforts continue to improve our techniques and methodology. Current work suggests that a change in the objectives for this particular outcome may be warranted and the team will suggest an alternative approach in the immediate future. Our laboratory is currently assessing possible transcriptional analysis programs can be used in these studies. At the end of this set of experiments we will have a relative abundance of each transcribed gene within the bacterium cultured in fish or in laboratory medium. The initial analyses of transcriptional activity will be repeated three times in order to define the reproducibility of the analyses.

Results
Several students have developed excellent skills in the manipulation of nucleic acids collected from the cells.

4. Associated Knowledge Areas

KA Code	Knowledge Area
135	Aquatic and Terrestrial Wildlife
311	Animal Diseases

313	Internal Parasites in Animals
712	Protect Food from Contamination by Pathogenic Microorganisms, Parasites, and Naturally Occurring Toxins

Outcome #2

1. Outcome Measures

Develop targeted intervention strategies to prevent pathogen contamination in bivalve rearing systems

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual
2013	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Research is needed to attempt to stabilize production in shellfish hatcheries by reducing occurrence of larval mortalities in shellfish by Vibrio tubiashii, through development of targeted intervention strategies aimed at preventing pathogen contamination in bivalve rearing systems i.e. by developing sensitive detection systems. Here, we propose to increase our understanding of the molecular mechanisms underlying the pathology of bacillary necrosis as well as to provide the shellfish industry with genetic markers which will help them transform hatchery bacterial populations from an uncontrolled to a controlled variable, thus stabilizing production at a consistent and productive level.

What has been done
We have determined the pathogenicity of strains of Vibrio tubiashii for larvae of mussels and oysters and found that a threshold of about 1000 cells per ml was necessary to cause adverse effects. We were also successful in determining the effects of probiotics in controlling the pathogenicity of Vibrio tubiashii for oyster larvae. The probiotics included algal species, baccterial strains and various seawater treatments. We found that sterilizing seawater increased the survival of Vibrio tubiashii, perhaps by eliminating competing bacterial species.

Results
Our research suggests that hatchery operators should maintain concentrations of Vibrio tubiashii below 1000 cells per m l to avoid outbreaks and that it is best not to attempt to sterilize seawater entering the hatchery because this could lead to greater growth of Vibrio tubiashii in the hatchery. It is best to maintain a "balanced" natural flora of bacteria in seawater to com bat outbreaks of Vibrio tubiashii.

4. Associated Knowledge Areas

KA Code	Knowledge Area
135	Aquatic and Terrestrial Wildlife
311	Animal Diseases
313	Internal Parasites in Animals
314	Toxic Chemicals, Poisonous Plants, Naturally Occurring Toxins, and Other Hazards Affecting Animals
712	Protect Food from Contamination by Pathogenic Microorganisms, Parasites, and Naturally Occurring Toxins

Outcome #3

1. Outcome Measures

To study mechanisms of important bacterial diseases affecting food sources in meat production.

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual
2013	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Clostridium perfringens type A food poisoning (FP) ranks as the third most commonly reported food-borne illness, affecting more than 250,000 individuals annually and causing economic losses of over $120 million in the USA. The food industry is interested in developing spore-inactivation alternatives to conventional heat processing technologies, including pressure-assisted thermal processing (PATP) approved by FDA as a novel food sterilization process, meeting consumer expectations for increased food safety, extended shelf life and improved food quality. Although the application of high pressure (HPP) (400 to 800 MPa) has been reported to inactivate pathogenic and spoilage bacteria, the inactivation of bacterial spores has been a major challenge as spores are extremely resistant to pressure.

What has been done
Alternative strategies to kill bacterial spores using pressure and temperature allowed by commercially available high pressure processing units can be developed by the use of our spore germination knowledge on how to sensitize spores towards inactivation treatments. Such approaches include: i) adding to the food species-specific spore germinants (e.g., L-asparagine

and KCl (AK) for C. perfringens FP strain SM101); ii) using heat and/or pressure treatment as primary activation steps of spore germination; iii) providing time for a germination step, in which the activated spores senses the presence of specific germinants and germinate in ≤ 30 min at optimum temperature; iv) inactivating germinated spores by PATP. In our recent studies (12) we have shown that C. perfringens spores can complete their germination in ~30 min in the presence of AK and PATP could efficiently inactivate C. perfringens spores in poultry meat formulated with AK. However, the feasibility of this alternative novel technology for various C. perfringens strains and meat products have not been determined, as only one strain and one meat product was used in our previous study

Results
Until this information becomes available, it will be difficult to design and develop novel strategies to control C. perfringens growth in meat products and prevent GI diseases caused by C. perfringens. This study will: i) define optimal conditions for efficient inactivation of C. perfringens spores, and ii) identify the basis for further development of protocols to generate unique opportunities for high-quality and minimally-processed foods. Work to date involves culturing various C. perfringen strains from a variety of meat products to allow the study to move forward.

4. Associated Knowledge Areas

KA Code	Knowledge Area
704	Nutrition and Hunger in the Population
712	Protect Food from Contamination by Pathogenic Microorganisms, Parasites, and Naturally Occurring Toxins

Outcome #4

1. Outcome Measures

To create diagnostic approaches to characterize the genetic difference between bovine herpesvirus type 1 variants and vaccine strains.

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual
2013	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Bovine herpesvirus type 1 (BHV-1) is the causing agent of Infectious bovine rhinotracheitis (commonly called IBR or red nose). An efficacious BHV-1 vaccine has been in use for many

years, but recently BHV-1 vaccine related abortion has been reported in an increasing number of herds. We plan to establish strain specific single nucleotide polymorphism profiles to assist the diagnosis of BHV-1 infection and to develop diagnostic tools that can quickly differentiate BHV-1 strains in abortion
cases.

What has been done
We are currently working with Pfizer to sequence BHV-1 isolates associated with vaccine related abortion cases. There are 5 BHV-1 isolates that have been obtained from BHV-1 vaccine related abortion incidences. The 2010 MLV vaccine, virus isolates from abortion cases, and wild type reference BHV-1 from NVD will be all sequenced at the same time. Once the whole genome sequence available for different BHV-1 strains or isolates, it is possible to identify specific single nucleotide polymorphisms (SNP) that could differentiate specific strains or isolates from each other. The SNP will be analyzed by alignment of whole genome from different strains using Geneious software. Once BHV-1 unique SNP profile is determined for each stain or isolate, the SNP profile of each virus can be used to identify specific strain by Sanger sequencing of PCR amplicons.

Results
A preliminary analysis of available BHV-1 gene sequences has demonstrated nucleotide variation is conserved between strains, suggesting strain specific SNP markers do exist (Table 1 and Fig 1). In addition to the SNP profile, a specific SNP of pathogenic strain could also be identified with the whole genome sequence comparison. These strain-specific SNP and pathogenic SNP will be useful for disease diagnosis and surveillance program, which is important for food security.

4. Associated Knowledge Areas

KA Code	Knowledge Area
311	Animal Diseases

Outcome #5

1. Outcome Measures

Develop strategies to increase immunity, including the development of vaccines, against pathogens that impact food sources. Identify the role of mother cow immunization on calf protection agains MAP.

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual

2013	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Mycobacterium paratuberculosis (MAP) is the cause of a severe and chiefly fatal condition in ruminants. It is mainly acquired by oral route in young calves, following ingestion of contaminated milk or infectious stools. We have shown that MAP, when in milk, changes phenotype and becomes more infectious both in vitro (epithelial cells) and in vivo (mice). Additional studies showed that mother cow antibodies against MAP-binding/invasion proteins, will prevent MAP uptake into the intestinal mucosa in the oral infection model, and impact the development of disease. We will study whether inhibition of the interaction of those MAP surface proteins with the intestinal mucosa will partially or completely inhibit uptake of MAP by the intestinal mucosa.

What has been done
Our understanding of the basic biology and mechanisms of pathogenesis of MAP has unfortunately lagged behind that of other pathogenic Mycobacterium spp. It is well recognized that the ability to identify the route of invasion and the host-pathogen interactions at a molecular level is important for the future development of strategies to prevent MAP infections or to limit the spread of the infection. Similarly, the elucidation of gene products specific to in vivo growth holds great promise in identifying new antigens for diagnostics or vaccine development, as well as products essential to pathogenesis. Hence, we have begun the process of addressing this through the development and characterization of transposon mutant libraries for MAP, along with gene and genome scale-
analyses of the basic biology of the organism and its interaction with the host.

Results
Current results have revealed significant information, which has allowed for better understanding of the mechanisms of entry. We also have identified an important regulator of MAP genes encoding for key proteins associated with invasion of the intestinal mucosa. MAP0482 (LuxR) is upregulated significantly when MAP is exposed to raw milk. Exposure to raw milk is associated with a significant increase in MAP invasion of bovine epithelial cell. Now, it is necessary to characterize the roles of the proteins in infection models.

4. Associated Knowledge Areas

KA Code	Knowledge Area
311	Animal Diseases
312	External Parasites and Pests of Animals

Outcome #6

1. Outcome Measures

Strategies to increase immunity in animals through dietary supplementation of selenium.

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual
2013	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Se is an essential micronutrient in cattle, and Se-deficiency can affect morbidity and mortality. Provision of adequate Se is important to prevent Se-responsive diseases in growing cattle such as nutritional myodegeneration and Se-responsive unthriftiness [reviewed Koller, et. al., 1983]. Many parts of the world, including Oregon and many states in the South of the USA, are known to have soil conditions conducive to deficient forage-Se content, potentially leading to clinical signs of Se deficiency in livestock grazing or fed crops raised on them (Stevens, et. al., 1985). Although the essentiality of Se has been known for five decades, the most effective method of Se delivery to cattle for optimum performance is still being investigated.

What has been done
Based on our preliminary data with the UPJOHN J-5 BACTERIN? vaccine, we hypothesize that higher Se in the diet may enhance adaptive immune responses. The CD4+ T helper cells (Th) orchestrate both cell-mediated and humoral immune responses and are necessary for the establishment of immunological memory following vaccination. Therefore, we anticipate that a Se-enriched diet will enhance Th cell functions leading to enhanced adaptive immunological responses.

Results
In order to determine if Th cell responses are in fact enhanced by Se supplementation, we will isolate peripheral blood Th cells from animals by magnetic separation.

Autologous cultures of leukocytes are pulsed with inactivated virus particles present in the Bovi-Shield GOLD® 5 vaccine (BVD types 1 and 2 and BRSV). Professional antigen presenting cells (APC) within the leukocyte cultures will engulf and process the viral proteins into short peptide sequence that will bind to MHC class II molecules expressed by APC. The peptide-MHC class II complex is the ligand for viral specific Th cells, and when isolated T cells are added in excess to antigen-pulsed APC, viral specific T cells will respond by releasing the cytokine IL-2. We have measure IL-2 levels by either a standard ELISA protocol or by intracellular IL-2 staining followed by flow cytometry (to specifically identify the T cell subsets producing IL-2). We hypothesize that an increase in IL-2 concentrations in calves fed Se- enriched diets correlates with the increased antibody titers we have previously observed

4. Associated Knowledge Areas

KA Code	Knowledge Area
311	Animal Diseases
314	Toxic Chemicals, Poisonous Plants, Naturally Occurring Toxins, and Other Hazards Affecting Animals

Outcome #7

1. Outcome Measures

Strategies to increase immunity in animals through development of vaccines against influenza.

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Action Outcome Measure

3b. Quantitative Outcome

Year	Actual
2013	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Vaccination provides protection from severe disease caused by influenza virus. The current influenza vaccine production system, i.e. embryonated chicken eggs, is considered suboptimal in capacity for production of annual seasonal influenza virus vaccine, and ineffective in several respects for production of vaccines against highly pathogenic avian influenza virus. Thus, there is an immediate need for methods to enhance the production of influenza virus vaccine from chicken eggs, and mammalian cell lines being considered for vaccine production.

What has been done
We expect to ascertain a PPMO which will enhance the production of influenza virus in chicken eggs and potential vaccine cell lines. A potential caveat in this project is the translation of the human siGENOME findings in A549 cell lines across to avian (embryonated chicken eggs). This is likely to be impacted by issues of conservation of gene sequence, gene function, and variations between species in redundancy of individual gene function. The availability of the chicken and canine genome sequences, and associated gene expression datasets, will enable bioinformatic filtering of PPMO candidates. It is also possible that the impact the inhibition of a single gene will not be sufficient to markedly increase virus production. In this case, more than one gene will be targeted by PPMOs. Regardless, the results from the proposed study will provide very useful preliminary results for the feasibility of PPMO-based approach for future grant applications.

Results
This project is just beginning with preliminary results to be reported in our annual report for 2014.

4. Associated Knowledge Areas

KA Code	Knowledge Area
311	Animal Diseases

Outcome #8

1. Outcome Measures

To evaluate the toxicity of various mycotoxins in food.

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual
2013	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Mycotoxins are secondary metabolites produced by fungi which reside in our food supply and on every surface in our environment and have been associated with a myriad of adverse health effects. Neurological and developmental effects from mycotoxin exposure have been reported but the mechanism(s) responsible for eliciting these toxic responses remain elusive. Moreover, little research has been done evaluating the toxicological effects of multi-mycotoxin exposure, which is generally how humans and animals are exposed to these toxins. Therefore, we propose to evaluate the developmental toxicity of four groups of the most predominantly detected mycotoxins in food (aflatoxins, fumonisins, ochratoxins and trichothecenes), both individually and in combination with each other, using the zebrafish (Danio rerio) model. Uncovering some of the mechanisms of toxicity that mycotoxins utilize to exert their effects can help design more specific studies investigating how to prevent mycotoxins induced diseases.

What has been done
A major goal is to define the full concentration response to each of the input chemicals by calculating the No Observed Adverse Effect Level (NOAEL) and EC100 for each produced endpoint (morphological and behavioral). Initially, we have defined the NOAEL and EC100

concentrations in our 5D tropical wild type strain of zebrafish. We hypothesize that chemicals that produce toxicity at higher concentrations may produce non- obvious, subtle effects and lead to important adult functional deficits at lower concentrations. We will initiate all exposures 6 hours post fertilization (hpf) with dechorionated embryos and sustain them continuously until 120 hpf.

Results
Preliminary results will be reported in 2014. We plan to assess of a battery of 18 body morphology endpoints at 5 days post fertilization (dpf) from high-resolution digital images. We have developed our own imaging system based on a Nikon Eclipse Ti inverted scope with a fully motorized, PC-controlled stage. With our custom software, it can image 96 wells in less than 3 minutes. The system automatically uploads the images to our own 100TB server for archiving and recalls them via barcode identifier for visual scoring of the 18 endpoints. This system also captures video for assessing cardiac endpoints. We have established a long track record of productive, rapid-throughout, phenotypic and behavioral screens in zebrafish (Mandrell 2012, Chen 2011, Truong 2011, Saili 2012, Tal 2012, Truong 2011, Harper 2011).

4. Associated Knowledge Areas

KA Code	Knowledge Area
311	Animal Diseases
312	External Parasites and Pests of Animals
313	Internal Parasites in Animals
314	Toxic Chemicals, Poisonous Plants, Naturally Occurring Toxins, and Other Hazards Affecting Animals
712	Protect Food from Contamination by Pathogenic Microorganisms, Parasites, and Naturally Occurring Toxins

V(H). Planned Program (External Factors)

External factors which affected outcomes

- Economy
- Appropriations changes
- Public Policy changes
- Government Regulations
- Competing Public priorities
- Competing Programmatic Challenges

Brief Explanation

All of the objectives and outcomes reported here are in the first year of a five year program. All research projects under this planned program have been initiated and we expect that we will have significant progress to report beginning with the 2014 annual report.

V(I). Planned Program (Evaluation Studies)

Evaluation Results

Establishment of food security involves many important aspects, from the source of the food to storage and transportation. We need to address gaps in our existing knowledge if

we are expected to create efficient ways to ensure food security to the broader population. The goal of this project is to develop a comprehensive approach to address infectious diseases and toxins in food animals. We will study infectious conditions and toxins that have impact on food sources. Better diagnosis of diseases as well as prevention and treatment measures are crucial for the establishment of safe source of nutrients.

Because the research described above has only just been initiated, we expect most evaluative results to be reported in our 2014 annual report.

Key Items of Evaluation

Although the spectrum of the proposed program seem broad, the main focus is to develop knowledge about infectious disease that affects the health of animals (food sources), establish diagnostic tests and attempt to progress towards creating new forms of therapy/prevention, such as vaccines and antisense morpholinos. The major NIFA outcome area to be addressed by this project is "Enhanced capacity of a sustainable global food system including new/improved plans, animals, technologies and management systems." Chosen indicators include: Improved animal genetics, Filed patents and licensing agreements, Increased efficiencies, Adoption of best practices and technologies.