CPS CENTER for PRODUCE SAFETY

CPS 2010 RFP
FINAL PROJECT REPORT

Project Title

Evaluation of amphibians and reptiles as potential reservoirs of foodborne pathogens and risk reduction to protect fresh produce and the environment

Project Period

January 1, 2011 – December 31, 2011

Principal Investigator

Michele Jay-Russell
University of California, Davis
Email: mjay@ucdavis.edu
Phone: 530-757-5756

Co-Principal Investigators

E. Rob Atwill, University of California, Davis, 530-754-2154, ratwill@ucdavis.edu
Robert Mandrell, USDA ARS, WRRC, 510-559-5829, Robert.mandrell@ars.usda.gov
Xunde Li, University of California, Davis, 530-752-0877, xdli@ucdavis.edu

Objectives

1. Determine if wild amphibians and reptiles are reservoirs of *E. coli* O157:H7 and *Salmonella* in the central California coast. We will identify the major species of amphibians and reptiles populating the leafy greens produce production environment and surrounding rangeland through intensive trapping and microbiological analysis.
2. Identify farm production practices, environmental factors and control strategies that reduce the risk of contamination from amphibian and reptile species in the leafy greens produce growing environment. Through statistical analysis, we will identify specific environmental factors (e.g, riparian habitat, wetlands, vegetation strips, ponds) and management practices that are associated with reduced pathogen prevalence. Molecular genotyping will be used to source track and provide a comparison of the genetic relatedness of strains from these animals with strains from waterways where they are collected, as well as comparison with our extensive database of human and environmental strains.
3. Extend knowledge of preventing produce contamination by amphibians and reptiles to the produce community. We will share the science-based knowledge gained from this study with growers, handlers, buyers, auditors, regulators, conservation groups, and other stakeholders to improve best practices relating to pre-season and pre-harvest environmental assessments and wildlife intrusion.

Michele Jay-Russell, University of California, Davis
Evaluation of amphibians and reptiles as potential reservoirs of foodborne pathogens and risk reduction to protect fresh produce and the environment

FINAL REPORT

Abstract

Our project measured the occurrence of *E. coli* O157:H7 and *Salmonella* among common species of wild amphibians (frogs, toads, salamanders, newts) and reptiles (lizards, snakes, turtles) in the central California coast produce production region and the Suwannee watershed in southeastern Georgia. Ten farms comprising 44 individual sampling sites were enrolled in California including 4 conventional and 3 organic produce farms, 2 adjacent wetland preserves, and 1 cattle ranch. Five mixed-produce irrigation ponds in Georgia were enrolled with assistance from collaborators at the University of Georgia. We collected and tested 1,444 and 510 samples in California and Georgia, respectively. *Salmonella* was cultured from several common species of amphibians (frog, toad, newt, salamander) and reptiles (snake, turtle). *Salmonella* was also cultured from non-irrigation waterbodies in California (natural and tailwater ponds, grassed ditch, wetland) and all 5 irrigation ponds in Georgia. *E. coli* O157:H7 was cultured from a single tailwater pond sample in California. Non-O157 shiga toxin-producing (STEC) strains were found in animals (frog, newt, toad, snake, lizard) and surface water in California including one irrigation reservoir sample. The highest concentration of generic *E. coli* was found in tailwater pond samples (mean 1,147 CFU/100 ml; range 0 – 12,080 CFU/100 ml). Irrigation reservoir samples in California had the lowest concentration of generic *E. coli* (mean27 CFU/100 ml; range 0 – 243 CFU/100 ml). The concentration of generic *E. coli* in water samples positive for foodborne pathogens ranged from 14 – 12,080 (mean 1,806) CFU/100 ml.
The findings underscore the importance of pre-season and pre-harvest environmental assessmentrelated produce safety practices, in particular those addressing animal intrusions and irrigation water quality.

Background

Human foodborne outbreaks and recalls associated with the consumption of leafy green produce contaminated with bacterial pathogens such as *E. coli* O157:H7 and *Salmonella* continue to occur despite efforts to improve microbial safety (Lynch 2009). This is in part the result of not having clearly identified the primary vertebrate sources of enteric bacteria for these commodities and extending that information to the produce growing agricultural community. Furthermore, the uncertainty surrounding the relative importance of different wildlife species and their habitat in the transport of foodborne pathogens has resulted in perceived conflicts between food safety and environmental goals, especially in the central California coast.

The body of knowledge concerning potential wildlife reservoirs of foodborne pathogens is growing, especially for large fauna (deer, elk, feral pigs), smaller mammals (coyotes, rodents), wild avian and insect (fly) species in pre-harvest contamination of fresh produce (Talley et al, 2009; Jay et al, 2007; Cooley et al, 2007). However, among the major groups of wildlife that may be present in the produce production environment, wild amphibians and reptiles are the least studied. There is epidemiological and experimental evidence that captive amphibians and reptiles can shed *Escherichia coli* O157:H7 and *Salmonella* and cause human illness (Austin and Wilkins, 1998; Parish, 1998; CDC 2003; Richards et al, 2004; Srikantiah et al, 2004; Mermin et al, 2004; Gray 2007). But, the relative significance of these cold-blooded species in the contamination of fresh produce or waterways in their natural habitat is unclear.

The overall goal of our project was to conduct research that will provide a science-based approach to reduce or eliminate bacterial contamination of leafy green and other fresh produce by amphibians and reptiles while minimizing negative impacts on native wildlife and their

habitat. We hypothesized that there are specific and identifiable combinations of environmental conditions, landscape features, and management production practices that influence the risk from amphibians and reptile intrusions into produce fields or nearby watersheds. We tested this hypothesis by conducting a prevalence survey of *E. coli* O157:H7, non-O157 shiga toxin-producing *E. coli* (STEC), and *Salmonella* in common amphibian and reptile species captured near produce production fields, wetland areas, and surrounding rangeland during the 2011 production period in the central California coast. Additionally, we collaborated with the University of Georgia (P. Adams, G. Vellidis) to evaluate foodborne pathogen prevalence in amphibians and reptiles during a concurrent Center for Produce Safety (CPS) study of mixed produce irrigation ponds in the Suwannee rivershed of southeastern Georgia.

Research Methods and Results

Field Sampling

Farm enrollment: Private properties with ponds or wetland areas in Monterey and Santa Cruz counties were enrolled confidentially and assigned an 8-digit alphanumeric farm code. Participants were recruited from existing studies in the central coast, and by advertising the study at conferences and in our monthly newsletter. Site selection was also done in consultation with wetland experts at the USDA Natural Resources and Conservation Service (NRCS). Likewise, in consultation with wildlife experts from Georgia, we selected five produce irrigation ponds already enrolled in another CPS study ("Science-based evaluation of regional risks for Salmonella contamination of irrigation water at mixed produce farms in the Suwannee watershed"). Field sampling was conducted from March to October 2011.

Trapping: Appropriate permits from wildlife agencies in California and Georgia were obtained to live-capture and release unlisted species of amphibians and reptiles. The protocol in both states was approved by the University Institutional Animal Use and Care Committees.

For each farm property enrolled, we first assessed the amphibian and reptile population and their habitat by visual observation. Based on findings from these visual surveys, and to minimize harm to the animals, we decided to use a combination of passive trapping and hand-catching the animals. Coverboards and PVC vertical pipe passive traps were set strategically at measured distances from produce fields, riparian corridors, holding ponds, stock ponds, and wetlands by using a range finder (Nikon Prostaff 550). Coverboards and PVC pipes take advantage of the natural tendency of amphibians and reptiles to seek refuge; unlike active traps, the traps do not need to be checked daily because the animals are free to enter and exit. Coverboards were left at the farms throughout the duration of the study and checked at least monthly. We used hand-grabs and hand-nets to catch terrestrial animals such as snakes, toads, and adult frogs around natural objects. Lizards were caught using a "noose" constructed by connecting fishing line to a solid pole. Aquatic animals such as tadpoles and frogs were also captured by using seine and dip nets during both day and night surveys. In addition, active trapping for turtles using baited hoop traps strategically placed in the irrigation ponds was done in Georgia.

Data Collection: A standardized data collection sheet was completed for each sampling event. If there was uncertainty regarding speciation of the animal, photographs and a description were sent to a professional herpetologist for identification. A YSI Water Quality Meter (Yellowsprings, OH) was used in the field to measure water temperature, pH, dissolved oxygen (%), and turbidity. A 100 ml water sample from each sampling period was sent to the UC Davis Analytical Chemistry laboratory for detection (mg/L) of ammonium, nitrates, total nitrogen, and

phosphorous. Climatic data (temperature, humidity, precipitation, etc.) were extracted from the UC Davis Statewide IPM Program.

Sample collection and transport: Cloacal swabs were collected and placed in liquid Stuart transport media (Copan Diagnostic Inc., Murrieta, CA). The swabs were inserted in the cloacae and rolled against the inner wall several times. A second swab, "ventral swab," was taken by rolling the swab from the cloaca to the neck. The animal was then placed gently into a sterile stand-up whirlpak bag containing 250-500 ml of phosphate buffered saline (PBS) for 10 minutes and allowed to defecate. Animals too small to be swabbed (<2 cm) were pooled in groups of 10 and placed in the PBS "bath." After processing, animals were released at the location where they were captured. At each site and sampling event, a paired one liter surface water sample was collected for microbiological and chemical analysis. In Georgia, water samples were processed by membrane filtration and the filter shipped to UC Davis.

Samples were kept in a cooler on ice during transport, stored in a refrigerator (4°C) upon arrival at the laboratory, and processed within 48 hours after sampling. Samples from Georgia were shipped overnight on ice to UC Davis; temperature was recorded upon arrival using a thermometer placed in the cooler.

Laboratory Methods

Validation studies: Prior to testing field samples, we validated our isolation method for *E. coli* O157 and *Salmonella* from amphibian/reptile and pond water samples in the laboratory. We also compared two pre-enrichment methods, tryptic soy broth (TSB) and universal pre-enrichment broth (UPB) as shown in Table 1. The results indicated that we could detect as few as 10 cells inoculated onto a cloacal swab, or per 100 ml PBS or pond water using for both bacterial species. For this study, we chose the TSB pre-enrichment method, which was recently published by our research group (Gorski et al, 2011).

Pre-Enrichment: Swabs were pre-enriched by placing them into 50 ml TSB and incubating for 2 hours at 25°C with sharking at 100 rpm then followed by 8 hours at 42°C with shaking followed by holding overnight at 6°C using a Multitron programmable shaking incubator. Animal PBS bath and surface water samples were filtered with a .45 nm filter membrane, which was then placed in 100 ml TSB broth then pre-enriched the same way as for swabs. A 1 ml aliquot of enrichment broth was store in the refrigerator at 4°C then shipped overnight on ice to the USDA ARS WRRC laboratory in Albany, California. The broths were re-enriched in TBS followed by testing for STEC and *Salmonella* as described previously (Cooley et al, 2007; Gorski et al, 2011).

Detection of *E. coli* O157: H7: After pre-enrichment, *E. coli* O157 was recovered by Immuno-Magnetic Separation (IMS) method using a Dynal Bead Retriever (Invitrogen, Carlsbad, CA) per the manufacture's instructions. After incubation and washing, 50 µL of the resuspended beads were plated onto Rainbow agar (Biolog, Hayward, CA) with novobiocin (20 mg/L) and tellurite (0.8 mg/L) (MP Biomedicals, Solon, OH) and streaked for isolation. The remaining 50µL will be plated onto Sorbitol MacConkey Agar (BD Becton, Sparks, MD) with cefixime (0.05 mg/L) (USP, Rockville, MD) and tellurite (2.5 mg/L) and streaked for isolation. The plates were incubated for 24 hours at 37°C. Two presumptive positive colonies on Sorbitol MacConkey Agar plate or Rainbow agar plate were selected for confirmation by PCR using a previously described method (Paton and Paton, 2003) with modification.

Detection of *Salmonella:* Ten µL of TSB bacterial suspension was added to 1mL of Rappaport-Vassiliadis (RV) (BD Becton, Sparks, MD) and incubated for 24 hours at 42°C. Five µL of RV

bacterial suspension will be streaked onto Xylose Lysine Deoxycholate (XLD) agar plates and incubated for 24 hours at 37°C for isolation. Two suspect colonies per positive plate were biochemically confirmed using Lysine, Simmons Citrate, Triple Iron Sugar and Urea reactions. We discovered during the study that some of the amphibian and reptiles contained a non-*Salmonella* with the same biochemistry profile. To address this issue, an indole test was added to the protocol. ARS isolates were confirmed by PCR to detect the *invA* gene.

For positive samples, up to six purified colonies were banked on cryobeads and stored at −80°C for further analysis.

Indicator Bacteria: We determined the presence and loads of indicator bacteria (generic *E. coli*) using the laboratory's routine membrane filtration method. For water and PBS samples, 50, 10, 5 ml are filtered through 47 mm, 0.45 and 956 µc pore size membrane filters. Filters were placed onto ChromEC agar plates for detecting *E. coli* and incubated for 2 h at 25°C followed by 8h at 42°C. Number of colonies in a plate within acceptable range (10-300 colonies per plate) were counted. Concentrations of indicator *E. coli* were calculated as No. CFU/100 ml.

Source tracking of *E. coli* O157:H7 and *Salmonella*: Pulsed-fieled gel electrophoresis (PFGE) analysis was used for fingerprinting of bacterial strains from amphibians, reptiles, and surface water samples. The PFGE was conducted according to the CDC's PulseNet standard procedure (Ribot et al., 2006). Briefly, bacterial isolates were retrieved from storage and suspended in cold buffer containing 1 M NaCl,10 mM Tris pH 8, and 10 mM EDTA for DNA isolation. DNA was digested in enzyme buffer with restriction enzyme XbaI. After digestion, DNA will be loaded into wells in agarose gel. The agarose gel was placed into the Electrophoresis Cell (Bio Rad) to run electrophori. After finishing the electrophori, the gel was stained with ethidium bromide, the image will be captured by Gel/Chemi Doc, and the image saved in computer as TIFF files using software Quantity I (Bionumerics). Images were analyzed and the similarity among different strains were characterized using computer software GelCompar II (Applied Maths).

Results

A total of 10 farms comprising 44 sites (4 conventional and 3 organic produce; 2 wetland preserves; 1 cattle ranch) in California and 5 mixed-produce irrigation ponds in Georgia were enrolled in the study from March through October 2011. We collected and tested 1,444 and 510 samples in CA and Georgia GA, respectively. Where multiple sample types (e.g., cloacal and ventral swabs, PBS "bath") were taken from individual animals, pathogens were recovered more often from PBS baths compared with swabs.

Detection of *Salmonella*

In California, *Salmonella* was cultured from 11 (3.3%) of 331 frog, 1 (5%) of 20 toad, 1 (20%) of 5 newt, 0 of 6 salamander, 23 (60%) of 39 snake, 7 (12%) of lizard, and 16 (13.6%) of 118 nearby waterbodies (Table 2). The prevalence was higher among reptile (30.6%) compared with amphibian (3.6%) species. At the California sites, *Salmonella* was recovered from non-irrigation water sources including grassed ditch, natural pond, river, tailwater (sediment) pond, and wetland slough water. In contrast, *Salmonella* was not detected in water samples from pre-irrigation reservoirs, but was isolated from 14 (8.2%) frog and snake samples collected at these reservoirs in the central coast (Table 3).

In Georgia, *Salmonella* was cultured from 15 (37.5%) of 40 toad, 23 (18.4%) of 125 turtle, 2 (40%) of 5 bivalve, and 10 (38.5%) of 26 water samples (Table 5). Unlike California, the overall prevalence of *Salmonella* in amphibian (15.6%) and reptile (18.4%) species was similar, although no snakes or lizards were tested in Georgia. *Salmonella* was detected in all

five irrigation ponds with a range of 3.2 – 7.7% in water and 1.6 – 22.6% in amphibian/reptile samples from individual ponds (Table 6).

Detection of *E. coli* O157 and non-O157 STEC

E. coli O157:H7 was cultured from a single tailwater pond sample in California; all other animal and water samples were negative for this strain in both states (Tables 2 and 5). Non-O157 STEC was isolated from three species (coast garter snake, western toad, rough skinned newt), natural pond water, and an irrigation reservoir sample in California (Tables 2); we did not culture for non-O157 STEC in Georgia samples.

Characterization of *Salmonella* strains

We unexpectedly found *Salmonella* strains from amphibian and reptile samples with atypical colony morphology on XLD plates. In order to confirm and identify these strains, we conducted additional tests. We confirmed that some of these unusual strains belong to *Salmonella enterica* Group III (Arizonae), a serogroup associated previously with amphibians and reptiles in captivity and linked to human outbreaks from fecal-oral contact with these animals. Approximately 10% of presumptive isolates were not confirmed as *Salmonella*, a significantly higher false-positive rate compared with similar ecological studies in the central California coast. Preliminary results from PFGE analysis suggest that some isolates from water and amphibian/reptile cluster into genetically related groups. Additional studies are underway to compare strains from this study with strains isolated during other ecologic studies in these two produce production regions (Gorski et al, 2011; Rajabi et al, 2011).

Environmental Parameters

In California, the highest concentration of generic *E. coli* was found in tailwater pond samples (mean 1,147 CFU/100 ml; range 0 – 12,080 CFU/100 ml) as shown in Table 4. Likewise, the highest dissolved oxygen (DO), ammonium, nitrate, and total nitrogen were found in runoff water from irrigation ditches and tailwater ponds. Irrigation reservoir samples had the lowest concentration of generic *E. coli* (mean 27 CFU/100 ml; range 0 – 243 CFU/100 ml). The generic *E. coli* concentration in water samples was not closely correlated with foodborne pathogen presence (mean 1,806, range 14 – 12,080 CFU/100 ml).

In Georgia, because water was pre-filtered for shipping to California, *E. coli* concentrations could not be determined for their irrigation ponds. However, we plan to compare the two states in a subsequent analysis using generic *E. coli* and water quality data from a parallel irrigation Suwannee watershed study conducted during the same time period (G. Vellidis, personal communication). A complete statistical analysis of relationships between microbiological, environmental and management practices will be provided in the final publication.

Outcomes and Accomplishments

The science-based data from this study fills a gap in knowledge related to the potential for wild amphibians and reptiles in proximity to produce production fields and waterbodies to serve as reservoirs of foodborne pathogens. The robust dataset compiled during this research can be used to develop co-management strategies to promote both food safety and environmental goals. We also demonstrated a strong collaboration between investigators at multiple institutions (UC Davis, University of Georgia, USDA ARS Western Regional Research Center) and industry in two different produce production regions of the United States. Furthermore, the research team successfully leveraged multiple projects to maximize the use of available resources and outcomes.

Summary of Findings and Recommendations

This is the first survey of foodborne pathogen occurrence in wild amphibians and reptiles in two diverse produce production regions in the United States. The findings will help inform the industry on potential reservoirs of zoonotic foodborne pathogens, and good agriculture practices to prevent microbial contamination from animal sources.

We confirmed that common wild amphibian and reptile species in the central California coast and southeastern Georgia may shed *Salmonella*, but *E. coli* O157 and other STEC strains are rare. In California, *Salmonella* was not detected in pre-irrigation reservoirs where positive frogs and snakes were identified. This suggests that the animals are not contaminating the water, although longer-term studies are needed to confirm this finding. In contrast, *Salmonella* was isolated from both toads and turtles in pre-irrigation ponds in Georgia, and similar genotypes were identified. Notably, irrigation water stored in reservoirs is sourced from wells in California, whereas water from the ponds in Georgia originates from rain and surface water sources including irrigation runoff, which may increase the risk of these ponds serving as disease reservoirs (Rajabi et al, 2011).

It is worth noting that the microbiological and chemical water quality parameters of water collected from tailwater (sediment) ponds and grassed ditches were potentially lower compared with other water sources at the California sites. Specifically, higher concentration of indicator bacteria (generic *E. coli*), dissolved oxygen, and chemicals (ammonium, nitrate, nitrogen) were found in these waterbodies. This may reflect the ability of these constructed sites to capture eroded soil and runoff, which has proven beneficial to the environment in agriculture settings (Tate et al, 2006; Knox et al, 2008). Additional studies are underway between UC Davis and NRCS to examine the ability of tailwater ponds and sediment basins to concentration foodborne pathogens in multiple produce production areas across the United States (R. Atwill, personal communication). In combination with our findings, the results may suggest improved management strategies to contain and remove *Salmonella* and other pathogens from farm environments, and reduce the risk of contamination of fresh produce due to runoff waters.

In summary, the findings from this study emphasize the need to continue to follow food safety practices, especially those relating to animal intrusions and irrigation water quality. The results from this study will be shared with stakeholders in the produce industry, conservation, and regulatory communities. Specifically, the data can be used to improve pre-season and pre-harvest environmental assessments and interventions as required in the Leafy Green Marketing Agreement metrics, in particular. In Georgia, more in-depth studies of *Samonella* occurrence in the Suwannee watershed are underway to better understand the ecology of these ponds and development of mitigation strategies. Wildlife isolates from this study will be shared with CPS investigators at University of Florida to compare genetic relatedness with their Suwannee watershed *Salmonella* strains to provide a better understanding of the ecology of *Salmonella* in these irrigation systems.

References

1. Austin, C. C., and M. J. Wilkins. 1998. Reptile-associated salmonellosis. J Am Vet Med Assoc 212:866-7.
2. CDC. 2003. Reptile-associated salmonellosis--selected states, 1998-2002. MMWR Morb Mortal Wkly Rep 52:1206-9.
3. Cooley, M., D. Carychao, L. Crawford-Miksza, M. Jay, C. Myers, C. Rose, C. Keys, J. Farrar, and R. Mandrell. 2007. Incidence and tracking of *Escherichia coli* O157:H7 in a major produce production region in California. PLoS One 2:e1159.

4. Gray, M. J., S. Rajeev, D. L. Miller, A. C. Schmutzer, E. C. Burton, E. D. Rogers, and G. J. Hickling. 2007. Preliminary evidence that American bullfrogs (*Rana catesbeiana*) are suitable hosts for *Escherichia coli* O157:H7. Appl Environ Microbiol 73:4066-8.
5. Jay, M., M. Cooley, D. Carychao, G. Wiscomb, R. Sweitzer, L. Crawford-Miksza, J. Farrar, D. Lau, J. O'Connell, A. Millington, R. Asmundson, E. Atwill, and R. Mandrell. 2007. *Escherichia coli* O157:H7 in feral swine near spinach fields and cattle, central California coast. Emerg Infect Dis 13:1908-11.
6. Knox, A.K. R.A. Dahlgren, K.W. Tate and E.R. Atwill. 2008. Efficacy of Natural Wetlands to Retain Nutrient, Sediment and Microbial Pollutants, Journal of Environmental Quality, 37:1837-1846.
7. Lynch, M., R. Tauxe, and C. Hedberg. 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiol Infect 137:307-15.
8. Mermin, J., Hutwagner L., D. Vugia, S. Shallow, P. Daily, J. Bender, J. Koehler, R. Marcus, F.J. Angulo, and the Emerging Infections Program FoodNet Working Group. 2004. Reptiles, amphibians and human *Salmonella* infection: a population-based, case-control study. Clin Infect Dis 38 (Supplement 3): S253-S261.
9. Parish, M. E. 1998. Coliforms, *Escherichia coli* and *Salmonella* serovars associated with a citrus-processing facility implicated in a salmonellosis outbreak. J Food Prot 61:280-4.
10. Richards, J., J. Brown, T. Kelly, A. Fountain, and J. Sleeman. 2004. Absence of detectable *Salmonella* cloacal shedding in free-living reptiles on admission to the wildlife center of Virginia. J Zoo Wildl Med 35:562-3.
11. Srikantiah, P., J. C. Lay, S. Hand, J. A. Crump, J. Campbell, M. S. Van Duyne, R. Bishop, R. Middendor, M. Currier, P. S. Mead, and K. Molbak. 2004. *Salmonella enterica* serotype Javiana infections associated with amphibian contact, Mississippi, 2001. Epidemiol Infect 132:273-81.
12. Paton and Paton. 2003. Paton, A., and J. Paton. 2003. Detection and characterization of STEC in stool samples using PCR, p. 45-54. *In*: *E. coli* Shiga Toxin Methods and Protocols. Humana Press, Totowa, NJ.
13. Rajabi, M., M. J. Jones, M. Hubbard, G. Rodrick, and A.C. Wright. 2011. Distribution and Genetic Diversity of *Salmonella* enterica in the Upper Suwannee River. Int J Microbiol doi:10.1155/2011/461321.
14. Ribot, E. M., M. A. Fair, R.Gautom, D. N. Cameron, S. B. Hunter, B. Swaminathan, and T. J. Barrett. 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog Dis 1:59-67.
15. Talley JL, Wayadande AC, Wasala LP, Gerry AC, Fletcher J, DeSilva U, Gilliland SE. 2009. Association of *Escherichia coli* O157:H7 with filth flies (Muscidae and Calliphoridae) captured in leafy greens fields and experimental transmission of *E. coli* O157:H7 to spinach leaves by house flies (Diptera: Muscidae). J Food Prot 72:1547-52.
16. Tate, K.W., Atwill, E.R., Bartolome, J.W., and Nader, G.A. 2006. Significant *Escherichia coli* Attenuation by Vegetative Buffers on Annual Grasslands. *Journal of Environmental Quality,* 35:795-805.

APPENDICES

Publications and Presentations

Gorski, L., C. T. Parker, A. Liang, M. B. Cooley, M. T. Jay-Russell, A. G. Gordus, E. R. Atwill, and R. E. Mandrell. 2011. Prevalence, Distribution and Diversity of *Salmonella enterica* in a Major Produce Region of California. Appl. Environ. Microbiol.

Jay-Russell, M. T. Evaluation of amphibians and reptiles as potential reservoirs of foodborne pathogens and risk reduction to protect fresh produce and the environment. 2011. Poster presentation at the 2nd Annual CPS Produce Research Symposium, Orlando, FL.

Jay-Russell, M. T., J. Montfort, Y. Liu, S. Huang, L. Gorski, R. E. Mandrell, J. Wheeler, D. Reis, X. Li, E. R. Atwill. 2012. Zoonotic Risks from Amphibians and Reptiles. 25th Annual Vertebrate Pest Conference, Monterey, California (accepted).

Budget Summary

We spent $37,212 in salary ($40,813 allocated) and $2,992 in benefits ($11,099 allocated). We spent $52,688.68 ($52,688.88 allocated) for supplies, and all of the travel funds ($2,895.12 allocated), and $28,240 for the subcontract with USDA ARS Western Regional Research Center. There was an overage of $639.24 due to an employee starting later than originally budgeted.

Account #	Name	End date
V465154	CPS amphibians	12/31/2012

	Allocations	Expenditures	Balance
Salary	$40,813.00	-$37,212.00	$3,601.00
Benefits	$11,099.00	-$14,091.46	-$2,992.46
Supplies	$52,688.88	-$52,688.68	$0.20
Travel	$2,895.12	-$2,895.12	$0.00
Subcontract	$28,240.00	-$28,240.00	$0.00
Indirects	$6,787.00	-$6,756.50	$30.50
TOTALS	$142,523.00	-$141,883.76	$639.24

Tables and Figures

Table 1. Comparison of *E. coli* O157 and *Salmonella* recovery from swabs and pond water using two pre-enrichment broths.[a]

	***E. coli* O157**		***Salmonella***	
Sample type	**TSB method**	**UPB method**	**TSB method**	**UPB method**
10 cfu/swab	+	+	+	+
10 cfu/swab	+	+	+	+
10 cfu/swab	+	N/A	+	N/A
10 cfu/100 ml water	+	-	+	+
10 cfu /100 ml water	+	+	+	+
10 cfu/100 ml water	+	N/A	+	N/A
50 cfu/100 ml swab	+	+	+	+
50 cfu/swab	+	+	+	+
50 cfu/swab	+	N/A	+	N/A
50 cfu/water	+	+	-	+
50 cfu/100 ml water	+	+	+	+
50 cfu/100 ml water	+	N/A	+	N/A
100 cfu/swab	+	+	+	+
100 cfu/swab	+	+	+	+
100 cfu/swab	+	N/A	+	N/A
100 cfu/100 ml water	+	+	+	+
100 cfu/100 ml water	+	+	+	+
100 cfu/100 ml water	+	N/A	+	N/A

[a]TSB = tryptic soy broth; UPB = universal pre-enrichment broth

Table 2. Recovery of foodborne pathogens from wild amphibians, reptiles and associated waterbodies, central California coast, 2011.

Source	No. Tested	*Salmonella* (%)	*E. coli* O157 (%)	Non-O157 STEC (%)
Amphibians				
Bull frog	185	8 (4.3)	0	0
California slender salamander	6	0	0	0
Newt (intergrade)	1	1 (100.0)	0	0
Rough skinned newt	4	0	0	1 (25.0)
Tree frog	146	3 (2.1)	0	0
Western toad	20	1 (5.0)	0	5 (25.0)
Subtotal	362	13 (3.6)	0	6 (1.7)
Reptiles				
Coast garter snake	23	12 (52.2)	0	1 (4.3)
Gopher snake	2	1 (50.0)	0	0
Racer snake	1	1 (100.0)	0	0
Ring neck snake	1	0	0	0
Santa Cruz garter snake	11	9 (81.8)	0	0
Sharptail snake	1	0	0	0
Southern alligator lizard	5	1 (20.0)	0	0
Western fence lizard	54	6 (11.1)	0	0
Subtotal	98	30 (30.6)	0	1 (1.0)
Other				
Crayfish	1	0	0	0
Water				
Water –pre-irrigation reservoir	19	0	0	1 (5.3)
Water – non-irrigation	99	16 (16.1)	1 (1.0)	6 (6.0)
Subtotal	118	16 (13.6)	1 (0.8)	7 (5.9)
Total	**579**	**59 (10.2)**	**1 (0.2)**	**14 (2.4)**

Table 3. *Salmonella* recovery from water, amphibian and reptile samples by location, central California coast, 2011.

			Salmonella (%)	
Location/produce types	**No. Sites**	**No. samples**	**Water**	**Amphibian/ Reptile**
Dry creek – apple orchard	1	9	0	0
Grassed ditch – brussell sprouts, cabbage, lettuce, fallow	3	14	1 (7.1)	0
Greenhouse	1	54	0	3 (5.6)
Irrigation reservoir – broccoli, herbs, lettuce, peas, fallow	6	171	0	14 (8.2)
Natural pond – berries, lettuce, fallow	7	147	4 (2.7)	21 (14.3)
Salinas River – celery, fallow	1	4	1 (25.0)	0
Tailwater pond – artichokes, asparagus, brassica, broccoli, cauliflower, celery, herbs, kale, lettuce, peas	17	139	8 (5.8)	3 (2.2)
Wetland/slough – grassland	8	41	2 (4.9)	2 (4.9)
Total	**44**	**579**	**16 (2.7)**	**43 (7.4)**

Table 4. Microbiological, physical, and chemical characteristics of waterbodies sampled on produce farms and conservation lands in the central California coast, 2011.[a]

Water type	No. sites	*Salmonellal* STEC	Generic *E. coli* (CFU/100 ml)	Temp °C	pH	DO (%)	Turbidity	Ammonium (mg/L)	Nitrate (mg/L)	Total Nitrogen (mg/L)	Phosphate (mg/L)
Grassed ditch	3	+/-	350	19.25	7.89	153.82	12.56	21.03	5.59	22.56	0.31
Greenhouse	1	-/-	776	24.65	9.82	90.175	22.23	5.63	0.25	1.54	2.23
Irrigation reservoir	6	-/+	27	18.87	8.0	125.82	6.56	9.91	2.77	11.34	0.10
Pond	7	+/+	173	17.52	7.99	118.76	9.64	2.51	0.55	1.27	0.15
Salinas river	1	+/-	71	22.34	8.67	152.3	8.86	5.73	2.2	7.36	0.2
Tailwater pond	17	+/+	1,147	19.65	8.23	141.24	17.50	17.72	7.9	21.83	1.28
Wetland/slough	8	+/-	424	18.15	8.023	109.51	37.82	16.65	8.20	7.84	3.21

[a]Values expressed as averages during the study period.

Table 5. Recovery of foodborne pathogens from wild amphibians, reptiles and associated waterbodies, southeastern Georgia, 2011.

Source	No. Tested	*Salmonella* (%)	*E. coli* O157 (%)
Amphibians			
American green tree frog	6	0	0
American green tree frog/southern toad	1	0	0
Dwarf salamander	5	0	0
Eastern spadefoot toad	23	9 (39.1)	0
Lesser siren	9	0	0
Pacific tree frog	2	0	0
River frog	5	0	0
Salamander (unspecified)	1	0	0
Southern cricket frog	4	0	0
Southern leopard frog	24	0	0
Southern toad	16	6 (37.5)	0
Subtotal	96	15 (15.6)	0
Reptiles			
Common musk turtle	7	1 (14.3)	0
Common snapping turtle	5	4 (80)	0
Eastern mud turtle	2	0	0
Florida softshell turtle	7	0	0
Red-eared slider turtle	104	18 (17.3)	0
Subtotal	125	23 (18.4)	0
Other			
Bivalve	5	2 (40.0)	0
Water			
Water –pre-irrigation pond	26	10 (38.5)	0
Total	**252**	**50 (19.8)**	**0**

Table 6. *Salmonella* recovery from mixed produce irrigation ponds, southeastern Georgia, March to October 2011.

		***Salmonella* (%)**	
Location/Produce type	**No. Samples**	**Water**	**Amphibian/reptile**
Pond 1 – fallow	13	1 (7.7)	1 (7.7)
Pond 2 – blueberries	84	3 (3.6)	18 (21.4)
Pond 3 – cucumbers, cotton	61	2 (3.3)	1 (1.6)
Pond 4 – eggplant, cotton	62	2 (3.2)	14 (22.6)
Pond 5 – eggplant, tomatoes	32	2 (6.3)	6 (18.8)
Total	**252**	**10 (3.9)**	**40 (15.9)**

Suggestions to CPS

None.