Center for Produce Safety 2010 RFP
Final Report due May 31, 2011

Project Title

Investigation of potential reservoirs of shiga toxin-producing *E. coli* and *Salmonella* in produce production areas of Arizona and Mexico

Project Period

October 15, 2010 – April 30, 2011

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Objective

The long-term goal of this project is to identify potential domestic and wild animal reservoirs of foodborne pathogens in leafy green production regions of Arizona and Mexico. The first phase of the proposed study will determine if free-roaming domestic and wild canids (e.g., coyotes) in these geographic regions shed *E. coli O157:H7*, and/or *Salmonella* in their feces.

Abstract
In May 2010, Romaine lettuce grown in Arizona was implicated as the vehicle in a multi-state outbreak of *E. coli* O145 infections. This is the first known leafy green-related shiga toxin-producing *Escherichia coli* (STEC) outbreak traced to the Yuma production region. Pre-harvest contamination was suspected, but the source of the outbreak was not definitively determined. The long-term goal of this project is to identify potential domestic and wild animal reservoirs of foodborne pathogens in produce production regions of Arizona and Northern Mexico. In the first "emergency response" phase of the project we are determining if free-roaming domestic dogs and coyotes in these regions shed STEC and/or *Salmonella enterica* in their feces. We focused on canids based on feedback from growers who reported that unleashed, free-roaming domestic dogs and coyotes are common in this region. Intrusions into produce fields have resulted in damage to leafy greens and other crops, but the food safety risk from stray dogs and coyotes is unclear. To address this gap in knowledge, we conducted a study during the 2010-2011 southwestern desert growing season to assess foodborne pathogen carriage in stray dogs and coyotes. In a unique industry-university partnership, the produce company managed the field activities including specimen collection and shipping, while UC Davis investigators conducted laboratory and data analyses. Three animal shelters were enrolled in the study and visited monthly to sample fresh feces from dogs recently impounded. Fresh coyote scat was collected at dawn by walking the dirt roads near produce fields. Samples were shipped from Yuma to Davis overnight and processed within 24 hours. Standard culture methods were used to isolate STEC and *Salmonella*. No shiga toxin-producing *E. coli* was identified among 473 samples, although 8 *E. coli* isolates belonging to serogroups O26, O145, or O157 were positive for other virulence determinants by PCR (eae, *hly*A) suggesting the potential to be a human pathogen. Overall, *Salmonella* was cultured from 33 (9.2%) of 358 dog fecal samples compared with 33 (32%) of 103 coyote scat samples. Twenty-nine *Salmonella* serotypes were identified including types that have been associated previously with human illness.

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* have been most frequently linked to the central California coast growing region of the United States. In May 2010, the CDC and FDA reported an outbreak of *E. coli* O145 involving 26 confirmed and 7 probable cases from 5 states (http://www.cdc.gov/ecoli/2010/ecoli_o145/); Romaine lettuce grown in Arizona was implicated as the vehicle. This is the first known leafy-green related shiga toxin-producing *E. coli* outbreak associated with the Yuma production region. A definitive source of contamination was not found during the environmental assessment, but pre-harvest contamination was suspected (http://www.fda.gov/Food/FoodSafety/FoodborneIllness/ucm235477.htm).

Our research team has studied extensively the potential vectors and transport mechanisms for *E. coli* O157:H7 dissemination in central coast counties (Monterey, San Benito), and found the bacteria in cattle, wildlife, water and soil samples at varying levels (Cooley et al, 2007; Jay et al, 2007; Jay et al, 2010). We now consider intrusion by wild or feral animals into crop fields or surrounding watersheds one of the significant risk factors for pre-harvest microbial contamination of leafy green vegetables. In contrast, reservoirs of foodborne pathogens in the Arizona and northern Mexico leafy greens production areas are not well-characterized.

According to reports from growers, populations of unleashed, free-roaming domestic dogs are a significant problem in desert growing regions of Yuma, Imperial, and Mexicali (Figure 1); stray

dog intrusions into leafy green produce fields have resulted in damage to the crops, but the food safety risk from these dog intrusions is unclear. It is worth noting that there is limited information in the literature on the prevalence of STEC infections among domestic dogs in the United States. A three-year study in Japan identified an extremely low prevalence of *E. coli* O157:H7 in dogs <1% (Kataoka et al, 2010). Researchers have reported a higher occurrence of *Salmonella* in dogs, ranging from 5% to over 70% prevalence, although most of this data comes from studies of dogs fed a raw meat diet (Joffe and Schlesinger, 2002; Lenz et al, 2009; McKenzie et al, 2010).

Growers have also reported coyote sightings and signs (tracks, scat) on roads adjacent to produce fields. Notably, our research team recently reported isolation of *E. coli* O157:H7 from 2 of 95 (2.1%) coyote fecal samples collected during a 2 year survey in the central California coast (Jay et al, 2010). Additional research is needed to determine if coyotes harbor these pathogens in other produce growing regions including Arizona and Mexico.

Research Methods and Results

Environmental Samples

The study was conducted during the 2010-2011 leafy greens production season in desert produce growing regions of the U. S. and Mexico. In cooperation with industry participants, we enrolled three animal shelters, which were visited approximately once per month to collect fresh fecal samples from recently impounded dogs. A standardized questionnaire was used to collect demographic data (e.g., location, date, dog breed, sex, age, etc.) from the facilities for statistical analyses. Additionally, wildlife samples (coyote scat, wild rodent scat, and snake/lizard carcasses) on the roads in and around produce fields were collected by walking the fields at dawn and collecting fresh feces from the ground or carcasses on glue board traps. Industry cooperators shipped samples overnight on ice to UC Davis. Samples were processed in the laboratory within 24 hours of collection.

Laboratory Analysis

Standard microbiological laboratory protocols were used to detect STEC and *Salmonella* including extended enrichment and immune-magnetic separation (IMS) (Cooley et al, 2007; Gorski et al, 2011). We also used a commercial IMS protocol to detect non-O157 *E. coli* serogroups. *E. coli* and *Salmonella enterica* species were confirmed by biochemical profiles and PCR; two colony picks from each positive sample were stored at -80°C.

E. coli strains were submitted to the Pennsylvania State University *E. coli* Reference Laboratory for confirmation of STEC serogroups using a multi-plex qPCR (DebRoy et al, 2011), and characterization of the virulence profiles (stx1, stx2, eae, *hly*A). *Salmonella* isolates were sent to the USDA National Animal Disease Laboratory at Ames, Iowa, for serotyping. USDA ARS Western Regional Research Center tested retrospectively frozen enrichment broths for the presence of stx1 and/or stx2 genes using qPCR.

Results

Descriptive Epidemiology

A total of 473 samples were collected by the industry cooperators, and tested at UC Davis during the 6-month study period. Samples included 358 (76%) domestic dogs and 115 (24%) wildlife (Table 1). *E. coli* O157 was isolated from 2 (1.9%) of 103 coyote scat samples, and none of the shelter dog samples; both strains were positive for *eae*, but negative for *stx*1, *stx*2,

and *hly*A virulence determinants (Table 2). All 71 non-O157 isolates belonging to serogroups O26, O103, O113, and O145 were also negative for *stx*1 and *stx*2, but 7 strains were positive for other virulence factors (Table 2). Frozen enrichment broths were negative for shiga toxin genes, which correlated with the culture findings.

Overall, *Salmonella* was cultured from 33 (9.2%) of 358 dog fecal samples compared with 33 (32%) of 103 coyote scat samples. *Salmonella* was also cultured from 3 (42.9%) of 7 snake/lizard carcasses captured on glueboards, but not from 5 rodent fecal pellets collected from traps adjacent to produce fields. Twenty-nine serotypes were identified including many that have been previously associated with human illness (Table 3). Approximately 60% of *Salmonella* strains cultured from dogs belonged to two dominant serotypes (Senftenberg and Typhimurium).

Conclusions

This is the first survey of foodborne pathogen occurrence in domestic and wild canids from a major desert production region in the US and Mexico. 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 found marked differences in the occurrence of pathogenic *E. coli*, which was rarely cultured, compared with *Salmonella,* which was relatively common in feces from shelter dogs and coyotes. Two shiga toxin-negative *E. coli* O157 strains were cultured from coyote scat samples and none from dog fecal samples. The low level of *E. coli* O157 in canids is similar to other studies (Jay et al, 2010; Kataoka et al, 2010). The public health significance of finding shiga toxin-negative EHEC serogroups (O26, O103, O113, O145, O157) in these shelter dog and coyote populations is unknown, although a few carried other virulence determinants (Table 2).

In contrast, *Salmonella* was cultured from 9.2% of shelter dog feces (range 3.2-14.9%) and approximately 1 of every 3 coyote scat samples collected in or near produce fields. We hypothesize that the higher rates of *Salmonella* recovery from free-roaming domestic dogs and coyotes may be due to their hunting and scavenging behavior. Notably, in a central California coast study, Gorski et al (2011) recovered *Salmonella* from only 3 (7.5%) of 40 coyote colonic fecal sample enrichment broths (Gorski et al, 2011). The higher recovery of *Salmonella* among coyote scat samples from the desert may be partially explained by differences in collection and laboratory culture methods. It is also possible that individual coyotes in this study were re-sampled since the animals were not captured and marked. Additional studies of wild-caught coyotes in desert produce production regions are needed to determine the actual prevalence in the population. Likewise, the sample size for other wildlife (snakes, lizards, rodents) was small, but the 3 positive snake/lizard carcasses caught in traps adjacent to produce fields indicates a potential risk of *Salmonella* shedding by these animals. Additional studies of reptiles in other regions of the US are underway, and should also be conducted in desert regions since these species are common in dry, hot environments.

In summary, the results suggest that canids in the US-Mexico desert production region are not major reservoirs of STEC, but may be a source of *Salmonella* contamination. The findings underscore the importance of good agriculture practices (GAPs) for leafy greens and other produce, especially those relating to animal intrusions and pre- and post-harvest environmental assessments.

Outcomes and Accomplishments

We successfully accomplished a rapid response to a recent lettuce-related outbreak through a unique industry-university partnership. The project benefited greatly from the cooperation of the industry, specifically management of the field activities including specimen and data collection and shipping. We also had excellent cooperation with county animal control shelters in Arizona and California, and government officials in Mexico. Arrangements for specimen transport across the border were made with USDA APHIS inspectors, and included carrying a letter from the Principal Investigator describing the purpose of the study and copies of permit exemptions from USDA APHIS and CDC.

Recommendations

- Continue to follow GAP metrics as described in the Arizona and California Leafy Green Market Agreements, especially those relating to animal intrusions and pre- and post-harvest environmental assessments
- Continue to follow *the Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables* relating to wildlife and animal intrusions
- Conduct a follow-up survey to determine *Salmonella* and STEC prevalence in other potential domestic and wildlife reservoirs in desert produce production regions
- Because of the high percent positive in coyote scat samples, subsequent surveys should attempt to determine the *Salmonella* concentration using a quantitative assay
- Compare domestic and wildlife *Salmonella* and STEC subtypes with strains from other environmental samples in the region (canals, irrigation water, soil amendments)
- Disseminate an English and Spanish language Fact Sheet on salmonellosis to animal shelters to prevent transmission among shelter animals and people (Appendix A)

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APPENDICES

Publications and Presentations (required)

Presentation at CPS Produce Research Symposium, Orlando, FL, June 28, 2011.

Budget Summary (required)

See UC Davis FIS2 report in file.

Tables and Figures

Table 1. Summary of *E. coli* O157:H7 and *Salmonella* results from domestic dog and wildlife fecal samples collected in a US-Mexico desert produce production region, November 2010 - May 2011.

	No. samples	*Salmonella* (%)	*E. coli* O157 (%)
Domestic dog			
Shelter 1	124	4 (3.2)	0
Shelter 2	100	9 (9.0)	0
Shelter 3	134	20 (14.9)	0
Total	**358**	**33 (9.2)**	**0**
Wildlife			
Coyote	103	33 (32)	2 (1.9)
Reptile	7	3 (42.9)	0
Rodent	5	0	0
Total	**115**	**36 (31.3)**	**2 (1.74)**
GRAND TOTAL	**473**	**64 (13.5)**	**2 (0.42)**

Table 2. *E. coli* serogroups and virulence factors found in domestic dog and coyote fecal samples collected in a US-Mexico desert produce production region, November 2010 - May 2011.

		Virulence Determinant		
E. coli **Serogroup (O antigen)**	**No. isolates**	***stx1*/stx2 (shiga toxin)**	***eae* (intimin)**	***hly*A (enterotoxin hemolysin)**
Domestic dog (n = 358)				
O26	3	0	1	1
O103	47	0	0	0
O145	5	0	4	0
O26 and O103	2	0	0	0
O103 and O113	3	0	0	0
Total	**60**	**0**	**5**	**1**
Coyote (n = 103)				
O26	1	0	1	0
O103	10	0	0	0
O157	2	0	2	0
Total	**13**	**0**	**3**	**0**
GRAND TOTAL	**73**	**0**	**8**	**1**

Table 3. *Salmonella enterica* serotypes isolated from domestic dog and wildlife fecal samples collected in a US-Mexico produce production region, November 2010 - May 2011.

Serotype	Source				CDC *Salmonella* Database*
	Domestic dog (n = 32)	Coyote (n = 33)	Snake (n = 2)	Lizard (n = 1)	
Aqua		1			Yes
Barranquilla		1			Yes
Derby	1				Yes
Drac		1			No
Duisburg		1			Yes
Enteritidis	2				Yes
Javiana		2			Yes
Livingstone	1				Yes
Mbandaka	1				Yes
Montevideo		1			Yes
Muenchen		2			Yes
Newport		3	1		Yes
Oranienburg	1				Yes
Sandiego		2			Yes
Senftenberg	14				Yes
Typhimurium	5	5			Yes
Typhimurium var 5-	1				Yes
II 47:b:1,5		1			Yes
III 17:z29:-		3			Yes
III 35:z29:-	1				Yes
III 42:(k):-			1	1	Yes
III 48:i:z		2			Yes
III 62:z36:-		1			No
III_40:z4,z32:-		3			Yes
III_48:g,z51:-	3				Yes
III_50:r:z		1			Yes
IV 44:z36:-	1	1			Yes
IV 47:l,v:e,n,x		2			No
IV Rough O:autoagglutinate	1				No

*Centers for Disease Control and Prevention (CDC) National *Salmonella* Surveillance Data: http://www.cdc.gov/ncidod/dbmd/phlisdata/*Salmonella*.htm

Figure 1. Photograph of a stray or "community" dog observed traveling along a canal in a major produce production region of Northern Mexico.