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FINAL PROJECT REPORT

Project Title
Contamination of leafy green crops with foodborne pathogens: Are wildlife a problem?

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Objectives
1. *Determine whether wildlife are a problem in contaminating leafy green produce fields with foodborne pathogens, and if so:*
2. *Estimate the magnitude of contamination of leafy green produce fields by three critical foodborne pathogens causing human illness,*

3. *Test hypotheses related to the spatial distribution of leafy green produce fields in relation to large areas of wildlife habitat,*
4. *Test hypotheses related to diversity and synanthropy of wildlife,*
5. *Use these results to identify wildlife species that have high potential for contaminating fields with foodborne pathogens,*
6. *Propose potential mitigation measures for producers to reduce or eliminate contamination of leafy green produce with foodborne pathogens carried by wildlife.*

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FINAL REPORT

Abstract

Microbial pathogen contamination of plant-derived foods can occur through multiple pathways, one of which is the introduction of pathogens into agricultural growing areas by wildlife. Leafy green crops are especially at risk because they, in some cases, can incorporate pathogens into their tissues and because they are often eaten raw by consumers. We examined wildlife incursion into leafy green crop fields in the San Luis Valley of Colorado to elucidate where these events occur, the wildlife species involved, and how many individuals were seen during incursion events. Data looked at incursion and pathogen contamination in relation to field distance to wildlife habitat and in relation to targeted mitigation efforts undertaken by the producer. Occupancy methods using camera traps and distance sampling detected occasional incursion by a wide range of species. Horned Larks (*Eremophila alpestris*) were the most common avian species encountered, and they were more abundant in crop fields further away from wildlife habitat than those near wildlife habitat. Camera data resulted in 1.7 million still images, and documented rodents, coyotes, and lagomorphs moving through fields. Additional occupancy analyses will be run when the entire image dataset has been identified. We also collected 361 wildlife fecal samples and 177 spinach and lettuce samples. Twelve of the wildlife fecal samples were presumptively positive for Shiga toxin-producing *Escherichia coli* but negative on subsequent confirmatory tests. None of the samples were positive for *Salmonella enterica* or noroviruses. The findings suggest that, in this study system, low levels of wildlife incursion do not necessarily directly translate to crop contamination, and rather than requiring omnibus restrictions and regulations across all growing regions to limit wildlife incursion into crops, we suggest developing regional restrictions based on differences in environmental conditions, agricultural practices, and risk of contamination by wildlife.

Background

Major foodborne pathogens cause approximately 9.4 million illnesses, 56,000 hospitalizations and 1,400 deaths each year in the U.S. (Scallan et al. 2011). As such, foodborne pathogens have serious economic consequences for public health as well as food producers who face product recalls when foodborne pathogen outbreaks occur from their products. Based on 4,589 foodborne disease outbreaks in humans caused by major pathogens, Painter et al. (2013) estimated that 46% and 22% of the 9.4 million illnesses were caused by consumption of produce in general and leafy vegetables, such as lettuce and spinach, respectively; more illnesses were attributed to leafy vegetables than any other commodity. Most (89.8%) of foodborne illness attributed to leafy greens are caused by five pathogens (data analyzed from Painter et al. 2013), and three of these—norovirus (59.3%), *Salmonella enterica* (14.1%), and Shiga toxin–producing *Escherichia coli* (STEC; 5.7%)—have either a documented or hypothesized zoonotic component that can involve transmission from wildlife to agricultural crops and people. Of particular concern is that both noroviruses and bacterial pathogens can be internalized by lettuce and spinach, either through the roots, through leaf stomata, or through structural damage to the plant (Saldaña et al. 2011, DiCaprio et al. 2012). This internal contamination cannot be removed during processing and allows foodborne pathogens to enter the human food chain.

Wildlife have recently become a concern for the spread of foodborne pathogens to vegetable produce fields, especially in leafy green crops (Langholz and Jay-Russell 2013). In general, the mechanism of contamination of leafy greens from wildlife is as follows: 1) wildlife carrying foodborne pathogens visit produce fields, 2) shed pathogens in their feces in the field,

and 3) contaminate surfaces of produce with pathogens, which can potentially become internalized in the plants (Saldaña et al. 2011, DiCaprio et al. 2012, Langholz and Jay-Russell 2013). While outbreaks of human illness have been attributed to wildlife contaminating produce fields with foodborne pathogens (Erickson and Doyle 2012), few studies have adequately documented the magnitude of wildlife contamination of produce fields, especially in terms of wild ungulates, birds, and medium-sized mammals such as raccoons (*Procyon lotor*) and coyotes (*Canis latrans*).

Of the three pathogens listed above, all but human noroviruses have been documented in wildlife (Jay et al. 2007, Atwill 2008, Gardner et al. 2011, Kilonza et al. 2013). Noroviruses cause primarily gastroenteritis in humans, are highly contagious, stable in the environment, and have a low infectious dose (Koopmans and Duizer 2004, Scipioni et al. 2008). Although human noroviruses (GII strains) have traditionally been considered strictly human pathogens, there is recent evidence that these strains can also be carried by domestic animals (Mattison et al. 2007, Summa et al. 2012), with the potential for carriage by wildlife. However, to our knowledge, wildlife have not been extensively examined for presence of human noroviruses, and this proposal offers a unique opportunity to explore the potential role of wildlife in norovirus maintenance and transmission. Of particular interest is the potential for canids, such as coyotes and red foxes (*Vulpes vulpes*), to transmit GII strains of noroviruses (Summa et al. 2012). *Salmonella enterica* is considered one of the most important human foodborne bacteria in industrialized countries, and wildlife have been pinpointed as a potential source of *Salmonella* contamination in leafy green crops (Hilbert et al. 2012). In a survey of samples taken from agricultural locations, Gorski et al. (2011) reported that wildlife samples had the highest *Salmonella* presence among sample types, including samples from soil, water, and cattle feces. Rodents trapped in agricultural fields were also found to be reservoirs for *Salmonella* and could play a role in its dissemination (Kilonzo et al. 2013). The role of wildlife in contamination of leafy green crops with STEC is also a concern. Multiple studies have suggested that both wild mammals and wild birds are capable of acting as reservoirs for *E. coli*; birds, in particular, may be able to move *E. coli* across long distances to, from, and among agricultural facilities (Van Donkersgoed et al. 2001, Havelaar et al. 2010, Carlson et al. 2011, Franklin et al. 2013). Wild ungulates are of particular concern with STEC contamination because of their association with a number of outbreaks in humans through contamination of crops and the environment (Cody et al. 1999, ProMED 2008, ProMED 2011). STEC has been found in relatively high incidences in elk and deer feces in Colorado (Franklin et al. 2013) and recently in antelope (Franklin, unpublished data).

In this study, we addressed the central question: Are wildlife a significant risk in contaminating leafy green produce? Although there is circumstantial evidence suggesting that wildlife may contaminate produce, the magnitude of the problem is poorly understood. For example, contamination of strawberries with *E. coli* O157:H7, which caused an outbreak in Oregon, was attributed to deer (*Odocoileus hemionus*), but transmission from deer to the field was not documented (Laidler et al. 2013). We conducted this study on lettuce and spinach crop fields in the San Luis Valley of Colorado, where wildlife, such as mule deer, pronghorn (*Antilocapra americana*), rabbits (*Sylvilagus* and *Lepus* spp.), black bear (*Ursus americanus*), and coyotes, have been opportunistically observed using leafy green fields.

Research Methods

Study Area

We conducted our research in the San Luis Valley in Saguache county of Colorado (Figure 1A). This area is the primary producer of leafy greens (lettuce and spinach) in Colorado, which is also the third largest producer of head lettuce in the U.S. (www.colorado.gov/cs/satellite/ag_markets/cbon/1251624936359).

In addition, the San Luis Valley is a mountain-basin landscape, which is a fairly typical agricultural landscape in the Western U.S., and shares similar characteristics to other leafy green production areas such as the Salinas Valley, California (Figure 1). These shared characteristics include surrounding wildlife habitat in the form of public lands, a well-developed riparian system, and nearby wildlife refuges (Figure 1). Thus, the San Luis Valley may serve as a model system with attributes that are shared with other leafy green production areas.

Leafy greens in the San Luis Valley are grown in circular, center-pivot irrigation fields approximately 790 m in diameter. Subsurface groundwater is used to irrigate these fields, which so far has been free of foodborne pathogens (Amy Kunugi, personal communication); this removes the confounding factor of indirect contamination of irrigation water by wildlife. In the San Luis Valley, planting dates for lettuce are 1 April – 10 July, and harvest dates extend from 15 June – 15 October, with the peak between 20 June and 20 September (NASS 2013).

General Design

We sampled 10 crop fields (8 planted in lettuce and 2 planted in spinach) from June through September, 2015. Fields were placed into two strata: fields in proximity to wildlife habitat and those not in proximity to wildlife habitat (Figure 2). Because of the large area covered by individual lettuce fields, each sampling unit was subdivided into 4 quadrants (Figure 3), which represented subsampling units. To determine the magnitude of wildlife contamination of leafy green fields, we initially planned to estimate four general metrics: 1) visitation rates by wildlife to produce fields, 2) density of wildlife visiting produce fields, 3) extent of fecal contamination of produce fields by different species of wildlife, and 4) the extent of contamination of leafy greens in proximity to feces shed by wildlife.

Field Methods – Wildlife Visitation

We used estimation methods for wildlife visitation rates that account for imperfect detectability in sampling wildlife because wildlife species are never detected with perfect accuracy. Two primary approaches were used: distance sampling (Buckland et al. 2004) and occupancy estimation (MacKenzie et al. 2006). Distance sampling is used to estimate the density of wildlife populations based on line transects where the data collected are distances to objects being surveyed from lines to estimate the object density. Occupancy estimation models use information from repeated observations to estimate detectability as well as the parameter of interest such as presence/absence of wildlife in crops fields. Occupancy models can also incorporate numbers of observed animals to estimate abundance or density (MacKenzie et al. 2006). These methods are used extensively in studies on wildlife ecology but rarely have been applied in studies at the wildlife-agricultural interface (McClintock et al. 2010).

Distance sampling transects were conducted along the edge of each quadrant. Transects were conducted by a single observer between the hours of 5:30 and 10:00 am when birds were most active. For each survey, a single observer walked along the edge of the quadrant being sampled for an average of 31 minutes (range = 19 to 55 minutes) and recorded the distance, using a laser range finder, and angular direction to each bird seen as well as species and number. Only detections within the field portion of the quadrant were included. Data were analyzed using program DISTANCE 6.2 (Thomas et al. 2010).

Six Reconyx Model HC500 HyperFire infra-red cameras were equally spaced along the edge of each sampled quadrant to capture images of wildlife entering the fields (Figure 3). Cameras were oriented such that edges of the field were monitored. Cameras were set to take a still image every 5 minutes and whenever motion was detected by the camera in order to maximize detection of the range of species visiting fields. Cameras were placed in all 10 fields to run simultaneously. Dependent on the planting schedule, cameras were moved to a new quadrant within each field every 4 weeks.

Field Methods – Sampling for Bacterial Contamination

We collected fecal samples from the periphery of the 10 sampled fields using systematic searches. When animal tracks were encountered along the periphery of sampled quadrants, we followed these into the field and collected any deposited feces. For feces collected in the crop field, the nearest crop plant was also collected for laboratory analysis. When possible, fecal samples were identified to species or species group (Elbroch et al. 2012) and then collected for laboratory analysis. All fecal samples were placed in sterile Whirl-Pak bags and shipped overnight to the University of Wyoming for laboratory analysis.

To increase sample size, we also captured medium and small mammals and birds along the edge of crop fields between 23–30 September 2015. Rodents and lagomorphs were trapped using 40 Sherman and 20 medium and 10 large Tomahawk box traps placed along the periphery of quadrants in 2 fields planted with spinach. Sherman traps were placed every 15.5 meters, medium Tomahawk traps every 31.1 meters, and large Tomahawk traps every 46.6 meters. Traps were baited with oats and apples; large Tomahawk traps were also sprayed with apple juice as an additional attractant for lagomorphs. Feces were collected from within traps or using swabs or fecal loops from captured individuals. Mammals were marked with ear tags to prevent multiple sampling from individuals and then released. Birds were captured using mist nets and baited box traps and held briefly in small cages (≤15 min) to allow for defecation. If birds did not defecate while in the cages, they were swabbed cloacally to collect a fecal sample. Birds were banded with appropriately sized bands issued by the U.S. Geological Survey Bird Banding Laboratory. All fecal samples were placed in sterile Whirl-Pak bags and shipped overnight to the University of Wyoming for laboratory analysis.

In addition to fecal samples, spinach and lettuce samples were also collected from sampled fields and analyzed for bacterial contaminants.

Laboratory Methods – Bacterial Contamination

Fecal and crop samples collected in the field were analyzed for the presence of *Salmonella enterica*, STEC, and noroviruses. In this study, many of the fecal samples collected were less than 0.1 g. This necessitated adaptation of the microbiological methods to accommodate these small sample volumes. Briefly, all fecal and lettuce samples were suspended in buffered peptone water (BPW) (1:10 w/v) and gently agitated for 5 min. Prior to partitioning of these samples into two equal volumes for *S. enterica* and STEC selective enrichment, 200 µl to 500 µl of these samples were preserved in an equal volume of 20% glycerol.

For STEC enrichment, the lettuce and fecal homogenates were incubated at 37°C ± 1°C for 5 h, and then supplemented with Acriflavin-Cefsulodin-Vancomycin (ACV) to suppress growth of background microbiota. Following ACV supplementation, samples were enriched via static incubation for 18–24 h at 42°C ± 1°C. Enrichments were then streaked onto cefixime-tellurite Sorbitol MacConkey (CT-SMAC) and Levine's Eosine-Methylene Blue (L-EMB) agars and incubated at 37°C ± 1°C for 18–24 h to isolate presumptive STECs. Up to ten presumptive colonies were then streaked onto CHROMAgar STEC and incubated at 37°C ± 1°C for 18–24 h. Bacterial colonies displaying typical colony morphologies were preserved in glycerol for further testing. These isolates were then subjected to PCR analyses to detect *stx1*, *stx2*, and *uidA* genes. Briefly, isolates were grown overnight in brain heart infusion broth (BHI) at 37°C, and 1 ml of the overnight culture was collected for PCR analyses. Following centrifugation, bacterial pellets were washed using 1 ml of 0.85% NaCl, suspended in 1 ml of sterile water, and heated to 100°C for 10 min. Unlysed cells and debris were removed by centrifugation, and the DNA-containing supernatants collected and stored at -20°C. One µl of each template was analyzed via PCR for the presence/absence of *stx1*, *stx2*, and *uidA* genes (Feng et al. 2011). Detection of amplicons was performed via agarose gel electrophoresis.

For *S. enterica* enrichment, sample homogenates were allowed to stand for 1 h at room temperature prior to incubation at 35° ± 2.0°C for 24 ± 2.0 h. Following enrichment, 0.1-ml

aliquots were transferred into 10 ml Rappaport-Vassiliadis (RV) medium tubes and another 1 ml to 10 ml tetrathionate (TT) broth tubes. RV enrichments were incubated at 42° ± 0.2°C and TT enrichments at 43° ± 0.2°C for 24 ± 2 h. A loopful of each RV and TT enrichment was then streaked onto xylose lysine desoxycholate (XLD) agar, Hektoen enteric (HE) agar, and xylose lysine tergitol 4 (XLT-4) agar and incubated for 24 ± 2 h at 35°C. All colonies displaying similar colony morphologies to *S. enterica* were streaked onto XLT-4 agar one more time prior to downstream analyses to improve the selectivity of the method.

Human noroviruses were detected from preserved fecal homogenates using a method endorsed by the U.S. Centers for Disease Control and Prevention as described by Pang et al. (2005). Briefly, viral RNA will be extracted directly from feces using the MagaZorb Total RNA Mini-Prep Kit (Promega, Madison, WI). A multiplexed real time reverse-transcriptase PCR (RT-PCR) was then performed with reaction conditions standardized by Pang et al. using a CFX96 Touch Real-Time PCR Detection System (BioRad). This PCR protocol identifies norovirus genogroups I and II, which are most frequently associated with human illnesses (Pang et al. 2005). As mentioned, many of the fecal samples collected were less than 0.1 g. Because of this limitation in sample size, we were only able to utilize 200 µl of a 5% fecal slurry for the detection of noroviruses, thus it was critical to test the efficacy of the RT-PCR assay utilized in this study. This RT-PCR assay developed by Pang et al. (2005) for norovirus GI and GII detection was reported to detect as few as 1.5–15 copies of the target RNA, and thus should provide satisfactory sensitivity. However, an initial analysis of fecal samples spiked with synthetic norovirus RNA (both GI and GII) indicated that the RT-PCR assay was adversely impacted by inhibitors. Within a representative sample of RNA extracted from feces, the Cycle threshold (Ct) of norovirus GI and GII synthetic RNA detection was inhibited by ≥15 Ct and 8 Ct, respectively. This shift in Ct, referred to as inhibition delta Ct (iΔCt), was calculated according to the formula:

$$i\Delta Ct = \text{Ct of spiked sample} - \text{Ct of the control reaction}$$

where the control reaction is theoretically free of RT-PCR inhibitors. For a RT-PCR that is 100% efficient, a shift of 3.32 cycles in Ct is obtained when a 1-log difference in target concentration is observed. Thus, in this study, iΔCt for norovirus GI and GII was equivalent to a reduction in assay sensitivity of ≥4.5 logs and 2.4 logs, respectively. The observed levels of inhibition and the possibility for low viral loads within the fecal slurry necessitated additional studies to optimize the viral RNA extraction procedure to reduce potential RT-PCR inhibitors. Empirical optimization of RNA extraction resulted in RNA samples relatively free of such inhibitors. Through this optimization, iΔCt for the norovirus GI RT-PCR was reduced to 1.3 and iΔCt for the norovirus GII RT-PCR was reduced to 0.77. These levels of inhibition are equivalent to a reduction in detection sensitivity of ≤0.4 log, and were determined to be within acceptable limits for norovirus detection in the fecal samples.

Research Results

Wildlife Visitation Rates

Distance Sampling – We conducted 37 distance sampling transects on the 10 crop fields where each field was sampled 3–4 times within each of the months of June, July and August. We detected 246 birds from 13 species, with the majority (73.6%) of detections being Horned Larks (*Eremophila alpestris*) (Table 1). Horned Larks were also detected on the majority of transects (Table 1). Using distance sampling, we estimated densities based on all bird species combined and Horned Larks only. Under this analysis, the best supported detection model was a uniform detection function with a cosine series expansion for all species and a polynomial expansion for Horned Larks (Figure 4) when post-stratified by proximity to wildlife habitat. In both cases, models with a global detection function across stratum performed better than models with

stratum-specific detection functions. Number of birds using crop field quadrants was calculated based on the density estimates with the inference that these represented numbers using crop field quadrants in a 30-minute time period during the morning hours.

All species and Horned Larks in crop fields showed a similar pattern of higher abundance in crop fields further away from wildlife habitat (within the agricultural matrix) than those near wildlife habitat (Figure 5). Based on overall estimates (Figure 5), 5.4 birds/30 minutes (95% CI = 3.2, 8.9) used crop field quadrants (11.8 ha) in the morning hours in fields near wildlife habitat, while 9.5 birds/30 minutes (95% CI = 6.8, 13.3) used crop field quadrants in the morning hours in fields further from wildlife habitat. A similar trend was observed for Horned Larks: 4.2 birds/30 minutes (95% CI = 2.2, 8.1) near wildlife habitat, 8.9 birds/30 minutes (95% CI = 5.6, 14.0) further from wildlife habitat.

Camera Traps – We monitored wildlife incursion into agricultural fields for 16 weeks using 60 motion-activated cameras (6 cameras per field). This allowed for continuous monitoring of field edges and resulted in 1.7 million still images. To date, we have analyzed nearly 400,000 images and have identified 4,222 images with animal activity, a detection rate of 1.2% of all images. Wildlife detected include coyotes, jackrabbits, rodents (Figure 6, Figure 7), and others (Table 2). Images were captured using both the motion-activated setting and the time-lapse setting, demonstrating the utility of using both methods when a range of species are being detected. This robust experimental design represents a novel methodology for documenting wildlife in agricultural areas, and the resulting images offer the most detailed camera dataset available on wildlife incursion into leafy green crop fields. Additional occupancy analyses will be run when the entire image dataset has been identified. These additional analyses include a comparison of animal incursion by taxonomic group in fields that are further away from contiguous wildlife habitat compared with those near wildlife habitat. In addition, we will also compare wildlife presence/absence, as well as incursion rates, in fields with wildlife mitigation measures to fields without wildlife mitigation measures. These results will help inform producers what mitigation measures are useful and the most efficient use of these approaches in time and space.

Bacterial Contamination

We collected 361 fecal samples from wildlife, 174 from mammals, 184 from birds, and 3 from unidentified species from all 10 fields (Table 3). In addition, we collected 168 spinach and 9 lettuce samples from 6 of the 10 fields, which were analyzed at the University of Wyoming; 800 additional lettuce samples were collected and analyzed by Southern Colorado Farms. The majority of samples collected from mammals were from lagomorphs (rabbits) and rodents, predominantly Ord's Kangaroo rats (*Dipodomys ordii*) (Table 3). The majority of avian samples were collected from Passerines, predominantly from Horned Larks and unidentified passerine species.

There was no evidence for any *Salmonella* contamination in wildlife feces or crop samples; none of the samples yielded even presumptive positives. Although 12 of the wildlife fecal samples were positive for *E. coli*, none of the samples were positive for STEC. Samples positive for *E. coli* were from both mammalian and avian species (Table 3). Samples with presumptive positive STEC were from both mammalian and avian species (Table 3). Specifically, 12 of the wildlife fecal samples yielded presumptive STEC isolates; however, PCR analyses revealed that these isolates did not encode for Shiga-toxin genes. Thus, no STEC was identified. None of the 977 plant samples collected from crop fields were positive for either *Salmonella* or STEC. In total, 110 fecal samples were analyzed for the presence of norovirus GI and GII; 20 of these samples were processed using the viral RNA extraction method described by Pang et al. (2005). However, when these samples were spiked with synthetic norovirus RNA it was clear that matrix associated RT-PCR inhibitors adversely impacted detection. Thus, the

RNA extraction step was optimized to reduce RT-PCR inhibitors. The remaining 90 RNA samples were prepared using this optimized extraction method. Samples analyzed for noroviruses were collected from passerines (50.0%), lagomorphs (36.4%), rodents (8.2%), canids (1.8%), unknown species (1.8%), and crop plants (1.8%). Noroviruses were not detected within any of these samples.

Outcomes and Accomplishments

We found little evidence that wildlife are a problem in contaminating leafy green produce fields with foodborne pathogens in the San Luis Valley, Colorado. Although both spinach and lettuce crop fields were visited by a variety of mammalian and avian wildlife, we found no evidence that feces deposited by these species were contaminated with the three pathogens for which we tested. Thus, the magnitude of contamination of leafy green produce fields by these three critical foodborne pathogens causing human illness was apparently nonexistent. There are a number of factors that could explain this lack of evidence for contamination:

1. We did not collect sufficient samples to detect the pathogens of interest. However, the prevalence of these pathogens in wildlife would need to be very low to avoid detection. In addition, the number of samples collected was a function of fecal deposition in fields by wildlife, which suggests fecal deposition was relatively low.
2. The environmental and agricultural risk factors associated with other leafy green production areas were minimal in the San Luis Valley. Leafy green crops on Southern Colorado Farms are irrigated solely with well water, minimizing the potential for contamination from water. In addition, the climate conditions are different than the Salinas Valley, California, where outbreaks of STEC in leafy greens have occurred (Jay et al. 2007). As a high elevation mountain valley, the San Luis Valley is cooler during the growing season and receives substantially more solar radiation than the Salinas Valley (Figure 8). These may present gross climatic differences affecting bacterial persistence in the environment. The San Luis Valley also has half the cattle present and no presence of feral pigs when compared with the Salinas Valley (Table 4). The Central Coast region of California has almost half the feral pig population in California (Waithman et al. 1999) whereas feral pigs have not been observed in the San Luis Valley. Both cattle and feral pigs were implicated in the 2006 outbreak of *E. coli* O157 in spinach that affected 26 states and Canada (Jay et al. 2007).
3. The mitigation measures used by Southern Colorado Farms are sufficient to control problem wildlife, such as wild ungulates, that have a higher potential to contaminate crops with the pathogens of concern. Currently, Southern Colorado Farms uses wide buffer strips of barren soil, rodent control devices on the periphery of their fields, and electrical fences on the periphery of specific fields to exclude rodents and wild ungulates, such as antelope, from their crops.

We believe that the combination of factors 2 and 3 above may contribute most to wildlife being a minimal problem in contaminating leafy green crops in the San Luis Valley. However, this study was only based on a single year of sampling. If pathogen contamination by wildlife is episodic based on variation in environmental factors, then our study did not capture that variation.

Although we found no contamination with the pathogens we investigated, we did see visitation of crop fields by wildlife. Some avian species, such as Horned Larks, had higher numbers visiting crop fields that were further from blocks of wildlife habitat than fields bordering those areas. This is consistent with the ecology of some of these species, most of which are considered synanthropic. For example, Horned Larks are associated generally with areas of

bare ground and avoid areas of tall vegetation (Beason 1995). Thus, they are often associated with agricultural landscapes. Continued analysis of the images from our camera traps will further elucidate these relationships with other species.

Summary of Findings and Recommendations

Although we found that wildlife visited leafy green crop fields to varying degrees in the San Luis Valley, Colorado, we found no evidence of *Salmonella enterica*, STEC or noroviruses in the feces of wildlife in and around those fields. This may partly be due to the three points listed in the Outcomes and Accomplishments section above (sample size, minimal environmental and agricultural risk factors, and existing mitigation measures). However, our overall results suggest that there may be substantial differences between growing regions in terms of the potential for wildlife to contaminate crop fields. The findings also suggest that, in this study system, low levels of wildlife incursion do not necessarily directly translate to crop contamination. Current restrictions on the presence of wildlife feces in crop fields require excision of a substantial portion of crop, which causes economic hardships on producers. In addition, recent complimentary research suggests that some practices put in place to limit wildlife incursion, such as removal of surrounding vegetation, may actually increase contamination risk (Karp et al., 2015). Rather than omnibus restrictions and regulations across all growing regions, we suggest that one alternative is to develop regional restrictions based on differences in environmental conditions, agricultural practices, and risk of contamination by wildlife. Wildlife incursion leading to contamination of leafy green crops in the San Luis Valley of Colorado appears to be a low risk, albeit high consequence, event.

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APPENDICES

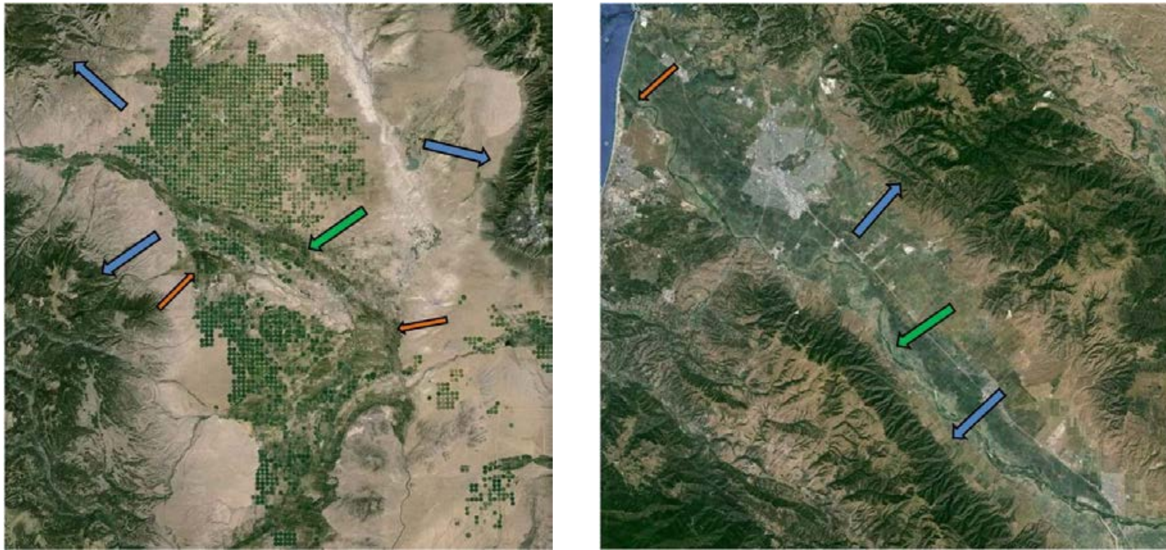
Publications and Presentations

PRESENTATIONS				
Date	Title	Venue	Location	Presenter
1 Dec 2015	Wildlife-borne Pathogens: Implications for Agricultural and Human Health	Colorado Ag Innovators	Fort Collins, CO	Alan B. Franklin
23 June 2015	Contamination of leafy green crops with foodborne pathogens: Are wildlife a problem?	Center for Produce Safety Research Symposium	Atlanta, GA	Alan B. Franklin
17 July 2015	Effects of Wildlife-Associated Pathogens on Food Security and Safety	USDA Deputy Under Secretary Elvis Cordova	Fort Collins, CO	Alan B. Franklin Sarah Bevins
22 April 2015	Effects of Wildlife-Associated Pathogens on Food Security and Safety	USDA Deputy Under Secretary Gary Woodward	Fort Collins, CO	Alan B. Franklin Sarah Bevins

Budget Summary

By the end of the award term there will be no remaining funds; some of the remaining funds are needed to cover publication costs and travel funds to attend the CPS Research Symposium in June 2016.

Tables and Figures



A. San Luis Valley, Colorado B. Salinas Valley, California

Figure 1. *Similar landscape characteristics between two leafy-green producing areas in the U.S.: (A) the San Luis Valley, Colorado and (B) the Salinas Valley, California. Blue arrows indicate public lands, green arrows indicate riparian systems and brown arrows indicate wildlife refuges.*

Figure 2. *Crop fields sampled from June through September in two strata in the San Luis Valley, Colorado: fields in proximity to wildlife habitat (blue circles) and fields not in proximity to wildlife habitat (orange circles). Red circles indicate crop fields operated by Southern Colorado Farms.*

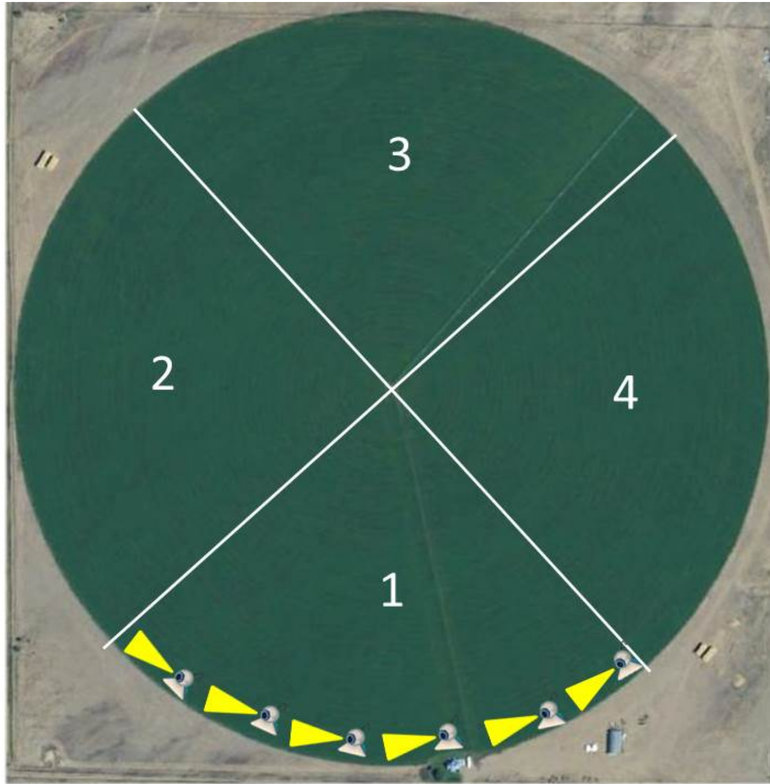


Figure 3. *Sampled crop field separated into 4 subsampling quadrants, which corresponds to the planting scheme used by Southern Colorado Farms. Yellow cones in quadrant 1 represent coverage of field edge by 6 camera traps, based on camera characteristics.*

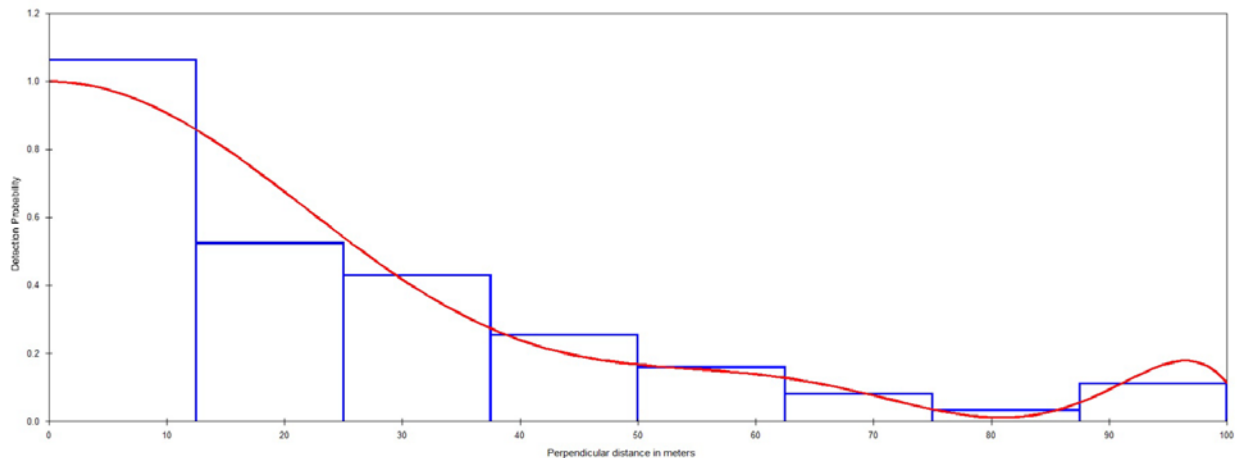


Figure 4. *Example of uniform detection function with polynomial expansion fit to observed detections for Horned Larks using distance analysis of line transects.*

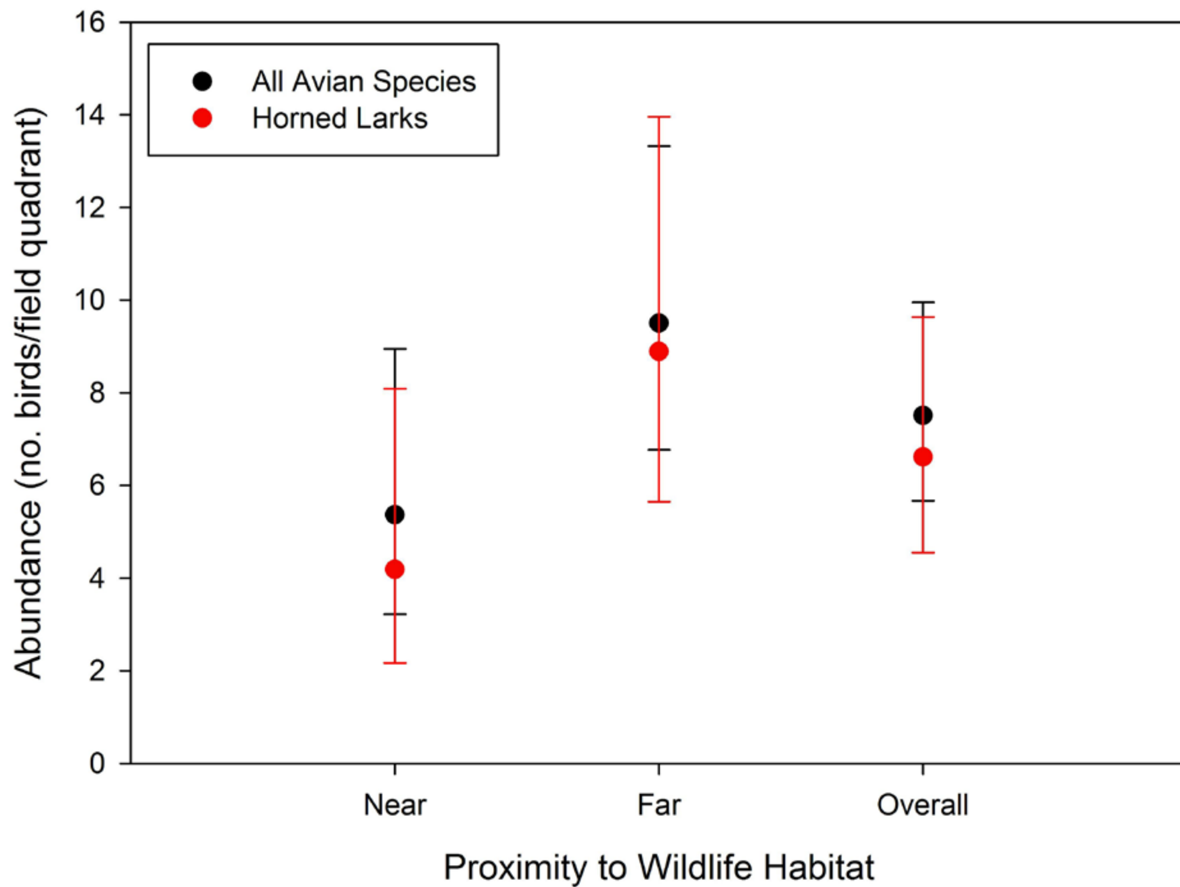


Figure 5. *Abundance, with 95% confidence intervals, of all avian species and Horned Larks based on distance sampling transects in fields near wildlife habitat and further from wildlife habitat (see Figure 2).*

Figure 6. *Select images from motion-detection/time-lapse cameras placed along field margins. Clockwise from upper left are, a coyote, jackrabbit, multiple jackrabbits in a single frame, and a kangaroo rat.*

Figure 7. *Coyote tracks showing movement through a lettuce field.*

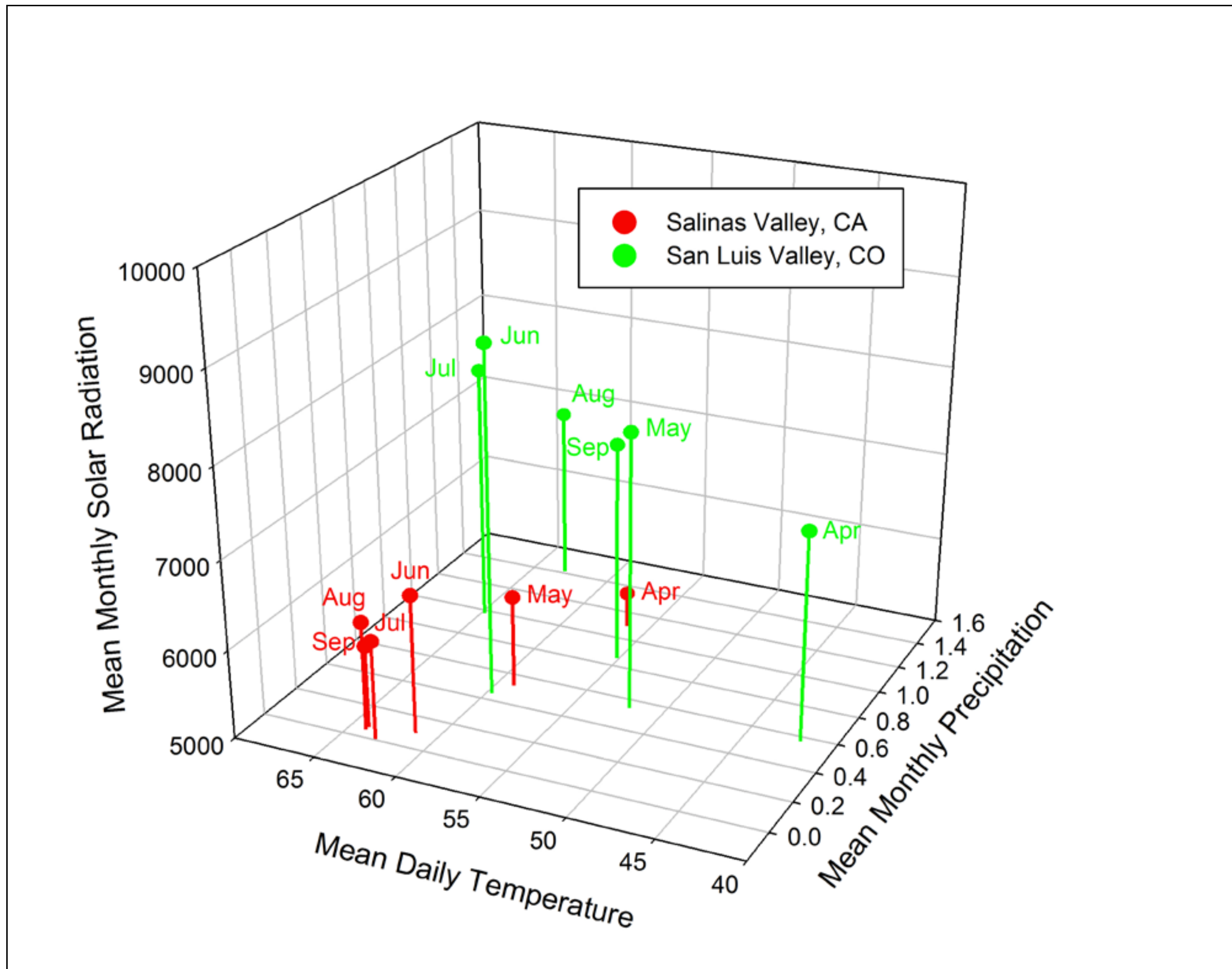


Figure 8. *Comparison of climatic environment during the growing season (April–September) between the Salinas Valley, California (red dots) and the San Luis Valley (green dots) from 1991–2010. Temperature and precipitation data are from the Western Regional Climate Center (www.wrcc.dri.edu) while solar radiation data are from the National Solar Radiation Database (rredc.nrel.gov/solar/).*

Table 1. *Bird species detected during 37 distance sampling transects in lettuce and spinach fields in the San Luis Valley, Colorado.*

Species	No. Transects (*n* = 37)	% Detections (*n* = 246)
Horned Lark (*Eremophila alpestris*)	28	73.6
Barn Swallow (*Hirundo rustica*)	6	8.5
Unidentified Passerine spp.	5	4.1
Rock Dove (*Columba livia*)	2	3.7
Brewer's Blackbird (*Euphagus cyanocephalus*)	1	1.6
Common Grackle (*Quiscalus quiscula*)	2	1.6
Mourning Dove (*Zenaida macroura*)	3	1.6
Red-winged Blackbird (*Agelaius phoeniceus*)	2	1.6
Killdeer (*Charadrius vociferous*)	1	0.8
Mallard (*Anas platyrhynchos*)	1	0.8
Western Kingbird (*Tyrannus verticalis*)	2	0.8
American Crow (*Corvus brachyrhynchos*)	1	0.4
Black-billed Magpie (*Pica hudsonia*)	1	0.4
Common Raven (*Corvus corax*)	1	0.4

Table 2. *Species detected in partial analysis of camera trap data from 60 cameras deployed over a 16-week period.*

Group	Species	Number of Images
Carnivores		
	Coyote (*Canis latrans*)	24
	Domestic Dog (*Canis domesticus*)	4
	Badger (*Taxidea taxus*)	1
	Gray Fox (*Urocyon cinereoargenteus*)	2
Lagomorphs		
	Jackrabbit (*Lepus* sp.)	188
	Cottontail Rabbit (*Sylvilagus* sp.)	129
	Unidentified	24
Rodents		
	Northern Grasshopper Mouse (*Onychomys leucogaster*)	6
	Ord's Kangaroo Rat (*Dipodomys ordii)*	182
	Unidentified	2775
Raptors		
	Red-tailed Hawk (*Buteo jamaicensis*)	2
	Unidentified	106
Doves		
	Mourning Dove (*Zenaida macroura*)	1
Passerines		
	Horned Lark (*Eremophila alpestris*)	67
	Barn Swallow (*Hirundo rustica*)	1
	Black-billed Magpie (*Pica hudsonia*)	9
	Unidentified	454
Plovers		
	Killdeer (*Charadrius vociferus*)	2
Corvids		
	American Crow (*Corvus brachyrhynchos*)	1
	Unidentified	26
Unidentified		211
Total		4215

Table 3. *Laboratory results for wildlife fecal samples collected in and around crop fields in the 2015 in the San Luis Valley, Colorado.*

Species	Samples		STEC Positive	
	No.	**%**	**Presumptive**	**Confirmed**
MAMMALS (n = 174)				
Canids	**5**	**1.4**		
Coyote (*Canis latrans*)	1			
Red Fox (*Vulpes vulpes*)	2		1	0
Unidentified Spp.	2			
Lagomporphs	**92**	**25.5**		
Black-tailed Jackrabbit (*Lepus californicus*)	13		1	0
Desert Cottontail (*Sylvilagus audubonii*)	3			
Unidentified Spp.	76		2	0
Rodents	**77**	**21.3**		
Deer Mouse (*Peromyscus maniculatus*)	9			
N. Grasshopper Mouse (*Onychomys leucogaster*)	9			
House Mouse (*Mus musculus*)	9			
Ord's Kangaroo Rat (*Dipodomys ordii*)	26			
Unidentified Spp.	24		2	0
BIRDS (n = 184)				
Raptors	**4**	**1.1**		
Burrowing Owl (*Athene cunicularia*)	2		2	0
Swainsons Hawk (*Buteo swainsoni*)	1			
Unidentified Falcon	1			
Doves	**5**	**1.4**		
Mourning Dove (*Zenaida macroura*)	2			
Rock Dove (*Columba livia*)	3		2	0
Passerines	**162**	**44.9**		
Black-billed Magpie (*Pica hudsonia*)	1		1	0
American Pipit (*Anthus rubescens*)	12			
Horned Lark (*Eremophila alpestris*)	41		1	0
European Starling (*Sturnus vulgaris*)	3			
Sage Thrasher (*Oreoscoptes montanus*)	1			
Red-winged Blackbird (*Agelaius phoeniceus*)	10			
Mountain Bluebird (*Sialia currucoides*)	1			
Western Kingbird (*Tyrannus verticalis*)	1			
Brewer's Sparrow (*Spizella breweri*)	2			
Savannah Sparrow (*Passerculus sandwichensis*)	2			
Vesper Sparrow (*Pooecetes gramineus*)	1			
White-crowned Sparrow (*Zonotrichia leucophrys*)	1			
House Sparrow (*Passer domesticus*)	1			
Unidentified Spp.	85		3	0
Unidentified	**13**	**3.6**		
UNIDENTIFIED (n = 3)		**0.8**		

Table 4. *Comparison of agricultural risk factors between the Salinas Valley, California and the San Luis Valley, Colorado.*

Location	Size (km^2)[a]	Cattle Density[b] (No./km^2)	Feral Pig Density[c] (No./km^2)
Salinas Valley	8,500	6.90	1.5
San Luis Valley	12,440	3.81	0.0

[a] Size of Salinas Valley represented by size of Monterey County and size of San Luis Valley by size of Alamosa, Rio Grande and Saguache Counties.
[b] Data from USDA 2012 Agricultural Census (agcensus.usda.gov).
[c] Based on estimates for the Rancho San Carlos, Monterey County, California, in Sweitzer et al. (2000).