



**CPS 2012 RFP  
FINAL PROJECT REPORT**

**Project Title**

Reducing the risk for transfer of zoonotic foodborne pathogens from domestic and wild animals to vegetable crops in the Southwest desert

**Project Period**

January 1, 2013 – December 31, 2014

**Principal Investigator**

Michele Jay-Russell  
Western Center for Food Safety – WIFSS  
University of California, Davis  
530-757-5756, mjay@ucdavis.edu

**Co-Principal Investigator**

Anne Justice-Allen  
Arizona Game and Fish Department  
623-236-7351, ajustice-allen@azgfd.gov

---

**Objectives**

- 1) *To determine if domestic animals and terrestrial and avian wildlife species in the desert southwest produce production region are reservoirs of shiga toxin-producing E. coli (STEC) or Salmonella.*
- 2) *To determine the extent to which wildlife and cattle share genetically related strains of STEC and Salmonella, and measure the movement of strains from livestock operations to produce fields by wildlife populations.*
- 3) *Extend knowledge of preventing produce contamination by domestic animals and terrestrial and avian wildlife populations to the produce and livestock communities.*

**Funding for this project provided by the Center for Produce Safety through:**

CDFA SCBGP grant #SCB12068

## FINAL REPORT

### Abstract

Vegetable fields are sometimes located in close proximity to concentrated animal feeding operations (CAFOs). Cattle housed in CAFOs may be reservoirs of *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* (STEC). However, little is known about the potential for wildlife that live at the CAFOs to transfer pathogens to nearby produce fields. We conducted a two-year study to evaluate if local livestock, terrestrial and avian wildlife populations in the desert southwest are reservoirs of *Salmonella enterica* and STEC, and to determine movement patterns of wildlife between CAFOs and produce fields. Fecal samples from cattle, wild birds and rodents were collected at least once each season during 58 sampling events that took place from March 2013 through November 2014 in southern Arizona and California. Wild birds were fitted with radiotransmitters and rodents with ear tags to determine movement patterns. Fecal and colon samples were collected from feral swine taken by USDA Wildlife Services in Arizona and New Mexico. Pathogens were isolated by pre-enrichment followed by immunomagnetic separation (STECs), selective plating, and PCR confirmation. A total of 750 cattle and 1,225 wildlife samples were analyzed. *Salmonella* was cultured from 6 wild bird species (brown-headed cowbird, common raven, great-tailed grackle, red-winged blackbird, white-crowned sparrow); 3 species were positive for non-O157 STEC (brown-headed cowbird, red-winged blackbird, white-crowned sparrow). *Salmonella* was cultured from 6 wild rodent species (Botta's pocket gopher, cactus mouse, desert pocket mouse, Merriam's kangaroo rat, round-tailed ground squirrel, western white-throated woodrat; one desert pocket mouse was positive for non-O157 STEC. No STEC O157 was isolated from wild birds, rodents, or rabbits. *Salmonella*, non-O157 STEC and STEC O157 were detected in cattle and feral swine samples. Cattle showed a significant peak in the prevalence of *Salmonella* and STEC during fall compared with spring and summer ( $p = 0.019$ ). Rodents and birds trapped at CAFOs did not show a significant seasonal difference in pathogen prevalence, but movement data indicated that birds traveled regularly between the CAFO and produce fields in southern Arizona. The majority of non-O157 STEC isolates did not encode all of the virulence markers associated with human infections, and did not belong to the "top 6" clinically serotypes. Growers should continue efforts to deter large flocks of nuisance birds from defecating in produce fields, and may need to increase the buffer zone distance from a leafy green field to a CAFO beyond 400 feet environmental assessments indicate a significant risk of bird activity in the field. The results from this study provide baseline data and information on risk factors that may impact the development of science-based best food safety practices in order to eliminate or minimize the risk of microbial contamination of fresh produce, while maintaining environmental stewardship.

### Background

In May 2010, an outbreak of *Escherichia coli* O145 infections was linked to Romaine lettuce grown in the desert southwest. This was the first known leafy green-related Shiga toxin-producing *Escherichia coli* (STEC) outbreak traced to the region. Based on initial investigations by the Center for Disease Control (CDC) and the Food and Drug Administration (FDA) following the outbreak, pre-harvest contamination was suspected, although no laboratory confirmed source was determined (FDA 2010).

According to reports from regional growers, wildlife intrusions into produce fields are common in the area. Frequent animal—most notably from dogs, javelina, and deer—sightings and signs (tracks, scat) in fields may be associated with damage and subsequent destruction of crops with economic losses. The food safety risk from these intrusions by wildlife is unclear, as limited information exists in the literature on the prevalence of foodborne pathogen infections among wildlife in the southwestern desert growing region. A recently published CPS Rapid Response study of domestic dogs and coyotes in the region conducted by our team during the 2010-2011 growing season found an STEC prevalence of 0% but *Salmonella* prevalences of 9.2 and 32.0% in dogs and coyotes, respectively (Jay-Russell et al. 2014). Wild bird and rodent intrusions into produce fields in the region are also of concern. An important migration and wintering stop for waterfowl lies approximately 25 miles northeast of Yuma, and the nearby Colorado River provides habitat to attract many bird species. The potential exists for birds to act as reservoirs of pathogenic bacteria or serve as vectors of produce contamination from nearby concentrated animal feeding operations (CAFOs), several of which are located in Yuma or surrounding counties.

In order to address these regional knowledge gaps, we conducted a two-year study to evaluate if local livestock, terrestrial and avian wildlife populations in the desert southwest are reservoirs of STEC and *Salmonella*. The results from this study provide baseline data and information on risk factors that may impact the development of science-based best food safety practices in order to eliminate or minimize the risk of microbial contamination of fresh produce, while maintaining environmental stewardship.

## **Research Methods and Results**

### Objective 1 Field Research Methods

Protocols for live-capture, handling, and release of wildlife were approved by the University of California, Davis Institutional Animal Care and Use Committee (IACUC protocol #17460). The Arizona Game and Fish Department had oversight of all wildlife trapping, and held a federal bird banding permit (#06613).

**Rodents and rabbits:** Agricultural areas with high levels of small mammal activity were identified by the presence of active burrows and tracks. Sherman traps (Sherman Trap product number XLK Folding Trap) were set at dusk 10m apart throughout the selected habitat, generally in a line formation, to trap small rodents, including mice, rats, and squirrels (Kilonzo et al. 2013). A total of 100 traps were set at a produce farm or CAFO on any given trapping night. Trapping took place over 3 to 5 consecutive nights every 3 to 4 months to enable seasonal comparisons, and traps were set at different farms each night to avoid recaptures. Traps were baited with 2 tablespoons of peanut butter mixed into a gallon size zipper locking plastic bag filled with cracked oats. We placed cotton balls or cotton batting in each trap as nesting material and to provide warmth overnight.

All traps were collected at sunrise and brought by truck to a nearby area with appropriate protection from the weather to process the animals. Each animal was removed from the trap by placing a zipper locking bag over the mouth of the trap, opening the door, and swinging the trap so as to collect the rodent in the bag. We recorded the species of each animal, and collected fecal samples directly from the individual into a microcentrifuge tube containing 1 mL phosphate buffered saline (PBS, Sigma Aldrich, St. Louis, MO) transport media. Fecal samples were also obtained from the contents of the trap, which were emptied into a Whirlpak bag.

Each animal was given two identification numbers: a field ID and an ear tag ID. The field ID number was written in permanent marker on the sample tubes and Whirlpak bags, and then the animal was ear tagged with a unique number using the appropriate sized ear tag applicator (National Band and Tag Company, product number 1005-1 for mice, product number 1005-3 for rats and squirrels). When all animals were processed, each was returned to its place of capture and released.

Samples were placed in a cooler with ice (not frozen) and shipped on blue ice to UC Davis Western Center for Food Safety/WIFSS lab for processing within 24 hours of sample collection.

**Small and medium size birds:** Mist nets were set approximately one hour before sundown in areas where birds were observed on a daily basis. Mist nets remained closed after they were set up, but were opened before sunrise. Poles for the mist nets were planted so the nets would be aligned in an east-west orientation, and were less likely to be seen by the birds as they flew through the area during sunrise. Nets were checked 20 minutes after the first flock of birds was observed in the area, and again every 20 minutes thereafter until most bird activity decreased or until the nets were too easily seen so birds could no longer be trapped, usually by 11am.

Birds caught in the mist nests were carefully removed and placed in pillowcases for transport. Aggressive birds, such as grackles, were placed in individual pillowcases, while other birds, such as brown-headed cowbirds, were placed in pillowcases together if they were caught in the same net. After all nets were checked, the captured birds were brought to a nearby area with appropriate protection from the weather to process them. We recorded the species of each bird, and whenever possible, collected fecal samples directly from the individual, either using a cloacal swab or by catching fresh droppings into a microcentrifuge tube.

Each bird was given a field identification number, which was written in permanent marker on the sample tubes, as well as a US Fish and Wildlife Service metal leg band number. The leg band was placed on the right leg of the bird and closed using the appropriately sized banding tool. Samples were placed in a cooler with ice (not frozen) and shipped to the lab for processing within 24 hours of sample collection. Birds were released directly from the processing area immediately after sample collection and leg banding.

**Ravens:** A decoy net launcher was set up in an area containing animal carcasses that was heavily visited by ravens. The decoy was set up one month before trapping actually began to acclimate the ravens to the presence of the equipment. On the trap day, the functional net launcher replaced the decoy one hour before sunrise, and animal carcasses were strategically placed to fall within the range of the net launcher. Some carcasses were opened up for easier access by the birds to bait them in. A technician laid in wait in a blind until the ravens arrived at which time the technician pushed a button that launched the net over the ravens, safely trapping them underneath. Once a raven was successfully removed from the net, sports tape was placed around the beak to ensure the safety of the technicians. We then collected fecal samples directly from the bird, either using a cloacal swab and/or by catching fresh droppings into a microcentrifuge tube.

**Feral swine and javelina:** USDA Wildlife Services specialists in Arizona and New Mexico collected colon and fecal samples from feral swine harvested during routine activities. UC Davis provided them with kits and instructions for collecting the samples. Wildlife Services

staff were instructed to tie off a 6 inch area of the colon with string. They then removed that section of the colon, keeping the contents intact, and placed it in a sterile zip-lock bag; and placed matching labels on the sample bag and data collection form where they provided the species, sex, and age of the animal, and the date and location where the animal was collected. The labeled bag containing the sample was then placed in a second clean bag, which was transported to UC Davis in a cooler with ice at 4C (not frozen) within 48 hours of sample collection.

Thirteen javalina samples collected during hunter harvest surveys in Yuma, Arizona in 2012 were included in the study for comparison and characterization of isolates.

**Cattle:** Cattle fecal samples were collected at least once each season from two CAFOs, one in Arizona and one in California. One sample was collected from each of five freshly deposited cow patties in a total of ten animal enclosures during each sampling session (n=50 per sampling session). A clean sterile scoop was used to place each sample in an individual sampling cup labeled with the enclosure number. Each cup was covered with a tight fitting lid and placed in a cooler with ice (not frozen) and shipped to the lab for processing within 24 hours of sample collection.

## Laboratory Methods

Pre-Enrichment: Swabs (BD, Sparks, MD) or 10 grams of feces were placed into 90 mL of tryptic soy broth (TSB) (BD, Sparks, MD). For rodent feces in a transport tube with 1 mL PBS, 9 mL of TSB was used for pre-enrichment. If the sample was trap content (e.g., cotton balls and feces), 50 mL of TSB was pipetted into the whirlpak bag containing the sample. Samples were then incubated for 2 hours at 25°C with shaking at 100 rpm followed by 8 hours at 42°C with shaking and holding overnight at 6°C using a Multitron programmable shaking incubator (Eppendorf, Hauppauge, NY).

Detection of STEC: For detection of STEC O157, immunomagnetic separation (IMS) using Dynal anti-E. coli O157 (Invitrogen, Grand Island, NY) beads was performed on TSB enrichment broths with the automated Dynal BeadRetriever (Invitrogen) per the manufacturer's instructions (Cooley 2014). 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). 50 µL of the resuspended beads were also plated onto MacConkey II Agar with sorbitol supplemented with 500 µl of potassium tellurite solution and 100 µl Cefixime (CT-SMAC); plates were streaked for isolation and incubated for 24 hours at 37°C.

To detect non-O157 STEC, pre-enrichment, broth was incubated in mEHEC selective media (Biocontrol, 12822 SE 32<sup>nd</sup> Street, Bellevue, WA) for 12 hours in 42°C followed by plating and incubating on Chrom STEC (DRG International Inc., 841 Mountain Avenue Springfield, NJ U.S.A.) Up to six presumptive STEC positive colonies were confirmed for presence of *stx1* and/or *stx2* genes by real-time PCR (Eppendorf, 102 Motor Parkway Hauppauge, NY). Confirmed STEC isolates were then characterized for virulence genes (*stx1*, *stx2*, *eaeA*, *hlyA*, *fliC* and *rfbE*) using conventional PCR. A subset of 36 strains were also tested by the Suslow Lab with STEC G2 combo (Roka Bioscience) for putative virulence markers (*ehxA*, *aggR*, *saaD* and *subAB*). Up to six suspect colonies per positive plate were confirmed by PCR (*invA*) and stored on a cryogenic media (TSB with 15% glycerol, Fisher Scientific, Pittsburgh, PA) at -80°C.

Selected isolates were submitted to the Pennsylvania State *E. coli* reference laboratory for O-serotyping.

Detection of *Salmonella*: After pre-enrichment, *Salmonella* was recovered by adding broth to Rappaport-Vassiliadis (RVS) (BD Becton, Sparks, MD) and incubation for 48 hours at 42°C as described previously (Gorski et al. 2013). A loopful of RVS bacterial suspension was then be streaked onto Xylose Lysine Tergitol 4 (XLT4) agar plates and incubated for 24 to 48 hours at 37°C for isolation. Up to six suspect colonies per positive plate were confirmed by PCR (*invA*) and stored on a cryogenic media (TSB with 15% glycerol, Fisher Scientific, Pittsburgh, PA) at -80°C.

### Objective 1 Results

Cattle, wild bird, and wild rodent/rabbit fecal samples were collected during 58 sampling events that took place from March 2013 through November 2014. We primarily focused our sampling efforts on two produce farms and two concentrated animal feeding operations (CAFOs) where we had the most trap success early in the project, and where the locations were most suited to addressing the objectives. During each trapping event, we set up as many as 6 mist nets and 2 ground traps for birds, 6 Tomahawk traps for rabbits, and 100 Sherman traps for rodents.

Trap success varied by location and bird versus rodent. Specifically, overall trap success for rodents was 33% with a 15% rate at CAFOs compared with 39% and 37% along roads and natural habitat adjacent to produce fields. In contrast, few birds were trapped in mist nets at the produce fields (<1 per net) compared with natural habitat adjacent to the produce field (4 per net) and on the CAFO (3 per net).

A total of 750 cattle and 1,225 wildlife samples were collected during the entire study period including 775 wild rodents, 383 wild birds, 1 wild rabbit, and 53 feral swine (Table 1). Diversity was highest among wild birds with 24 different species (Table 2) identified compared with 14 wild rodent species (Table 3). *Salmonella* was cultured from 6 wild bird species (brown-headed cowbird, common raven, great-tailed grackle, red-winged blackbird, white-crowned sparrow); 3 species were positive also positive for non-O157 STEC (brown-headed cowbird, red-winged blackbird, white-crowned sparrow). *Salmonella* was cultured from 6 wild rodent species (Botta's pocket gopher, cactus mouse, desert pocket mouse, Merriam's kangaroo rat, round-tailed ground squirrel, western white-throated woodrat; one desert pocket mouse was also positive for non-O157 STEC (Table 3). No STEC O157 was isolated from wild birds, rodents, or rabbits.

The overall prevalences for CAFO cattle samples were 6.9% *Salmonella*, 38.4% non-O157, and 17.3% STEC O157 (Table 1). Feral swine samples were also positive for *Salmonella* (6.9%), non-O157 STEC (7.5%) and STEC O157 (1.9%). Javelina prevalences were 15.4% for *Salmonella*, 7.7% for non-O157 STEC, and 0% STEC O157. Seasonal trends were observed with wild birds showing peak pathogen prevalence of *Salmonella* and non-O157 STEC in spring when they are most active, while rodents and cattle show a peak in *Salmonella* fecal shedding in the fall when cattle also show a peak in non-O157 STEC (Table 4; Figures 1-3). Cattle show a significant peak in the prevalence of *Salmonella*, *E. coli* O157 and non-O157 STEC during fall compared with spring and summer ( $p = 0.019$ ). Rodents and birds trapped at the CAFO do not show a significant seasonal difference in pathogen prevalence, but movement data do indicate that birds traveled regularly between the CAFO and produce fields as discussed below.

## Objective 2 Field Research Methods

**Rodent movement:** To determine rodent movement patterns, each animal was given two identification numbers: a field ID and an ear tag ID. The field ID number was written in permanent marker on the sample tubes and Whirlpak bags, and then the animal was ear tagged on the left ear with a unique number using the appropriate sized ear tag applicator (National Band and Tag Company, product number 1005-1 for mice, product number 1005-3 for rats and squirrels). When we trapped animals that we had already ear tagged previously, we were able to determine the location where they were originally ear tagged to determine if they moved between CAFOs and nearby produce fields.

**Bird movement:** In order to track movement patterns in birds, medium and large birds were fitted with nano coded radiotransmitters (Lotek, Ontario, Canada) at the Arizona CAFO (Figure 6). To ensure that the transmitter would not be a physical burden to the birds, birds were only fitted with a radiotransmitter if the transmitter weighed less than 3 percent of the bird's total weight (in most cases it was <5% of the bird's total weight).

Prior to catching any birds, we constructed the backpacks to hold the transmitters in place on the birds (Figure 5). We cut two 12-15 inch segments of Teflon (Bally Ribbon Company, 0.1 inch width for medium birds and 0.33 inch width for ravens) and sewed them together to make an X-shape. We ensured that the thread laid flat so as not to cause irritation to the bird. Then we cut the threads that made the top two pieces of the X-shape into sharp angles and inserted them into the top tube on the transmitter, entering from opposite directions so they crossed each other inside the tube. We pulled each of them out on the other side of the tubing. We then did the same for the bottom two pieces of the X through the bottom tubing on the transmitter.

After catching a bird, two people worked together to put the harness on the bird - one person held the bird while the other worked on securing the harness. We put the harness over the bird's head and pulled the wings through the straps, making sure to place all the birds' feathers on top of the Teflon so the harness could be tightened properly and so it wouldn't loosen over time. We also ensured that the top harness straps were sitting on the proximal side of the bone that sticks up on the shoulder so they wouldn't slide down. The person holding the bird held the sewn X in place on the breast bone while the second person tightened the harness because it's important for proper fit and placement that the X-shape stay exactly in place. We tightened the bottom straps first, then the top. We continued checking to make sure that the straps were tight enough and that the X-shape hadn't moved until we had reached the proper fit. After both people agreed that the harness was properly placed and tight enough, the second person tied off both ends of the harness using a secure knot and sealed the knots with eyelash glue. We waited 60-120 seconds for the glue to dry, and released the bird.

Birds were tracked using two receivers that were placed at the CAFO and two receivers that were placed in nearby leafy green fields (Figure 5 and 6). Whenever a bird wearing a backpack flew within 800 to 1000 meters of a receiver, their signal was picked up by antennae and the date, time, and their individual ID number was recorded. Each receiver was connected to a solar panel and 12 volt battery to ensure constant recording throughout the season. Half of the birds were fitted with backpacks in spring, while the others were deployed in fall to allow for a seasonal comparison. The medium sized birds' transmitters had a battery life of 3 months while the raven transmitters had a battery life of 6 months due to their larger size. The backpacks

were made so that around the time that the batteries stopped working, the backpack material would start to fray and fall off the birds.

## Objective 2 Results

A total of 27 of the ear tagged rodents were captured either once or twice again on future dates after their initial capture. None of the rodents were trapped in any locations away from their initial point of capture. In fact, some rodents were recaptured in the same or close to the same traps in the same order that they were trapped on their first trap date.

A total of 103 birds captured on the Arizona CAFO were fitted with radiotransmitters to track their movement patterns. The majority of these birds were red-winged blackbirds, *Agelaius phoeniceus* (n=66). Table 5 shows the number of each bird species that was fitted with a radiotransmitter during each season. As shown in Figure 5, preliminary analysis of bird movement from the CAFO demonstrates that birds frequently travel between the CAFO and beyond the 400 ft buffer zone, the minimum area away from livestock per current Arizona and California Leafy Green Marketing Agreement (LGMA) food safety guidelines.

## Virulence profiling

Serogrouping of 305 STEC isolates revealed 30 O-antigen types (Table 6). The majority of cattle (117/130) and feral swine (1/1) STEC O157 were serotype *E. coli* O157:H7 and encoded *stx1* and/or *stx2*, *eae*, and *hly* genes (Table 7). Among the non-O157 STEC isolates, almost 50% belonged to 6 serogroups (O136, O157, O171, O109, O2, and O160) and did not encode the *eaeA* gene (Table 8). Of note, only 14 (~5%) non-O157 STEC isolates (O103, O145, O26, O45) belonged to the “top 6” clinically relevant serogroups. Approximately 50% of a subset of isolates (n = 36) analyzed with Roka classified these as STEC based on more stringent criteria. One strain (feral pig) was found to carry a putative virulence gene (*saaD*). Additional molecular studies to source track isolates from wildlife and cattle samples are underway. Pulsed-field gel electrophoresis will be used to compare selected isolates from cattle and wildlife at different distances from point of collection.

## Objective 3 Outreach Methods and Results

Numerous presentations to stakeholders were given to audiences at scientific conferences and local industry workshops and meetings. Details of the presentations are provided in Appendix A.

## **Outcomes and Accomplishments**

We successfully trapped wild rodents and birds to collect fecal samples directly from these animals, and we were able to obtain fresh cattle fecal samples from two participating CAFOs in southern California and southern Arizona. We were able to collect samples often enough and in great enough quantity to allow for seasonal comparisons of prevalence and future modeling of risk factors. Additionally, the availability of expertise from Arizona Game and Fish Department biologists allowed us to apply wildlife capture-recapture and radio-telemetry techniques to a produce food safety project. We fitted wild rodents with ear tags and wild birds with radiotransmitters that were secured to their bodies with backpacks. We set up four receivers and six antennae in a total of four field locations, along with solar panels to ensure constant functioning. We maximized use of the funds and demonstrated the value of a multi-disciplinary



team in addressing co-management of agriculture production systems within a desert environment.

This research also builds on findings from a previous CPS Rapid Response project (Jay-Russell et al., 2014) and fostered continued close collaborations with the leafy greens industry in Yuma, Arizona and southern California. We utilized expertise from the UC Desert Research and Extension Center (DREC) in southern California and the University of Arizona Yuma Agricultural Center, thereby strengthening relationships with cooperative extension in both states. Through these relationships, we gained the trust and cooperation of growers and CAFO managers who helped us in accessing field sites and establishing receiver locations. The knowledge gained from the study was shared with stakeholders in multiple venues, especially within the southwestern desert (Appendix A).

The project also supported training and career development for a postdoctoral scholar, Dr. Paula Kahn-Rivadeneira, who was ultimately recruited to the University of Arizona, Yuma Agricultural Center during the second year of this grant. She is now a cooperative extension specialist in produce food safety and wildlife serving Arizona growers and ranchers. Despite the job change, she continues to work on this project, which is a close fit with her role as an extension specialist in Yuma. She will lead and co-author several manuscripts resulting from this work.

## **Summary of Findings and Recommendations**

- Desert fauna including wild rodents, birds, and javalina may serve as reservoirs of *Salmonella* and non-O157 STEC, but they do not appear to be significant sources of *E. coli* O157, even when in close proximity to high density cattle operations with documented fecal shedding of *E. coli* O157:H7. As shown in previous studies, cattle and feral swine fecal material is much more likely to contain *E. coli* O157:H7 compared with small mammals and avian species. However, due to the heat, feral swine are not found in the southern-most leafy green production areas of Arizona and California.
- Wild rodent home ranges are small enough that these animals are often captured in the same location on multiple dates. It is unlikely that rodents travel from CAFOs to produce fields if the distance between the two is further than a 0.5 hectare area or if there are substantial barriers between the two, such as a canal or major roadway. Therefore, it is highly unlikely that wild rodents transmit pathogens directly from CAFOs to produce fields.
- Rodent density was relatively low at the CAFOs, but high (>30% trap success) along leafy green field edges and adjacent habitat; additional studies should evaluate if the buffer (typically dirt roads between fields) between rodent habitat and produce is sufficient to minimize intrusions into the fields.
- Birds travelled regularly between CAFOs and distant sites, sometimes on a daily basis, and their flight paths sometimes take them directly over produce fields. Since birds do show seasonal variation in their prevalence levels of *Salmonella* and non-O157 STEC, growers are advised to deter birds from their fields during the growing and harvesting season.
- Bird density and diversity was highest at the CAFO and in natural habitat adjacent to produce fields. During the study, birds were rarely captured within fields suggesting that they spent most of their time between CAFOs and roosting areas. Future studies should focus on the specific attractants that cause birds to enter produce fields (e.g., irrigation, harvest, etc.) and target bird control resources to these higher risk activities.

- If leafy green fields are near CAFOs and experience significant bird intrusions (especially large groups of brown-headed cowbirds, ravens, grackles, blackbirds, and sparrows), the minimum 400 foot buffer zone between the field and the perimeter of a CAFO, recommended as an LGMA food safety practice, may need to be increased.
- The majority of STEC isolates obtained in this study were not among the “top 6” non-O157 serogroups (O26, O111, O103, O121, O45 and O145) and did not encode genes for intimin and other virulence markers typically associated with human infections. Rapid discrimination of clinical relevance would improve risk management decisions and, potentially, avoid unnecessary destruction of crops.

## References

Cooley, M.B., M. Jay-Russell, E.R. Atwill, D. Carychao, K. Nguyen, B. Quiñones, R. Patel, S. Walker, M. Swimley, E. Pierre-Jerome, A.G. Gordus, and R.E. Mandrell . 2013. Development of a robust method for isolation of Shiga toxin-positive *Escherichia coli* (STEC) from fecal, plant, soil and water samples from a leafy greens production region in California. PLoS ONE 8(6): e65716. doi:10.1371/journal.pone.0065716.

Crawford, W., M. Baloch et al 2010. Environmental Assessment: Non-O157 Shiga Toxin-Producing *E. coli* (STEC). Available at:

<http://www.fda.gov/Food/FoodSafety/FoodborneIllness/ucm235477.htm>

Gorski, L., M.T. Jay-Russell, A.S. Liang, S. Walker, Y. Bengson, J. Govoni, and R.E. Mandrell. 2013. Diversity of pulsed-field gel electrophoresis pulsotypes, serovars, and antibiotic resistance among *Salmonella* isolates from wild amphibians and reptiles in the California Central Coast. Foodborne Pathog. Dis. ahead of print. doi:10.1089/fpd.2012.1372.

Jay-Russell, Michele T. 2013. What is the risk from wild animals in food-borne pathogen contamination of plants? CAB Reviews 2013 8, No. 040.

Jay-Russell, M.T., A. F. Hake , Y. Bengson, A. Thiptara, and T. Nguyen. 2014. Prevalence and characterization of *Escherichia coli* and *Salmonella* strains isolated from stray dog and coyote feces in a major leafy greens production region at the United States-Mexico border. PLoS ONE 9(11) pub. Nov. 20.

Kilonzo C., X. Li, E.J. Vivas, M.T. Jay-Russell, K.L. Fernandez and E.R. Atwill. 2013. Fecal shedding of zoonotic food-borne pathogens by wild rodents in a major agricultural region of the Central California Coast. Appl. Environ. Microbiol. 9(20):6337-44. doi: 10.1128/AEM.01503-13.

## APPENDICES

### Publications and Presentations (required)

#### Presentations

Date	Title	Organization/ Conference	Location	Presenter
5 Mar 2014	Zoonotic enteric pathogens of wild boar, feral swine, and javelina (Order: <i>Artiodactyla</i> )	26 <sup>th</sup> Vertebrate Pest Conference	Waikoloa, HI	Michele Jay-Russell
5 Mar 2014	Poster: An approach to examining the wildlife and livestock interface with leafy green produce in the southwest desert	26 <sup>th</sup> Vertebrate Pest Conference	Waikoloa, HI	Paula Kahn-Rivadeneira
29 May 2014	The role of wildlife in the dissemination of E. coli O157:H7 and other STECs to fresh produce	Governor's Conference/STEC Coordinated Agricultural Project (CAP) Annual Meeting	Lincoln, NE	Michele Jay-Russell
25 June 2014	Reducing the risk for transfer of zoonotic foodborne pathogens from domestic and wild animal to vegetable crops in the southwest desert.	Center for Produce Safety Research Symposium	Newport Beach, CA	Michele Jay-Russell
27 July 2014	The wildlife-livestock interface in produce food safety	American Veterinary Medical Association Annual Convention (Featured Speaker)	Denver, CO	Michele Jay-Russell
31 July 2014	The role of wildlife in the transfer of enteric zoonotic pathogens from livestock to leafy green produce fields in the desert southwest	Wildlife Disease Association Annual Conference	Albuquerque, NM	Paula Kahn-Rivadeneira
21 Aug 2014	Working together to address animal intrusions and improve food safety in the Yuma Valley and beyond	Yuma Safe Produce Council Pre-Season Meeting	Yuma, AZ	Paula Kahn-Rivadeneira
4 Sep 2014	Reducing the risk for transfer of foodborne pathogens from animals to vegetable crops in the desert southwest	University of Arizona, Yuma Agricultural Center, Pre-Season Vegetable Workshop	Yuma, AZ	Paula Kahn-Rivadeneira
15 Sep 2014	Birds, rodents, cattle, and	University of	Tucson, AZ	Paula Kahn-

	salad, Oh My! Zoonotic pathogen risks associated with the livestock:wildlife interface near leafy green produce fields in the desert southwest	Arizona Soil, Water, and Environmental Science Department Colloquium		Rivadeneira
10 Oct 2014	Evaluating pathogen transfer between livestock and wildlife, and the resulting risk to leafy green produce in the southwest desert	University of Arizona Food Safety Consortium Annual Meeting	Tucson, AZ	Paula Kahn-Rivadeneira
9 Dec 2014	Species diversity of wild rodents and birds in and around leafy green produce fields - Which critters pose the most risk?	Pesticide Applicators Professional Association Continuing Education Meeting	Indio, CA	Paula Kahn-Rivadeneira
31 July – 3 Aug 2015	Poster: Spatial and temporal factors affecting prevalence of <i>Salmonella</i> and STEC in wild birds and rodents in proximity to CAFOs and vegetable fields in the Southwest desert	International Association for Food Protection Annual Meeting (submitted)	Portland, OR	Paula Kahn-Rivadeneira
31 July – 3 Aug 2015	Poster: Diversity of O-serotypes and virulence markers of STEC isolates from feedlot cattle and wildlife in the desert southwest	International Association for Food Protection Annual Meeting (submitted)	Portland, OR	Peiman Aminabadi

### **Budget Summary (required)**

All funds will have been spent by the end of the project, including travel costs associated with presentation of the Final Report at the CPS Symposium 2015.

**Tables and Figures (optional)**

Table 1. *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* (STEC) frequency in (N = 750) and wildlife (N = 1,225) samples, southwestern desert, 2013-2014.

<i>Salmonella</i>	Sample size	Number positive	Percent positive (%)
Cattle	750	52	6.9
Feral swine	53	15	28.3
Javelina*	13	2	15.4
Wild birds	383	8	2.1
Wild rodents/rabbits	776	24	3.1
Non-O157 STEC	Sample size	Number positive	Percent positive (%)
Cattle	750	288	38.4
Feral swine	53	4	7.5
Javelina*	13	1	7.7
Wild birds	383	10	2.6
Wild rodents/rabbits	776	1	0.1
STEC O157	Sample size	Number positive	Percent positive (%)
Cattle	750	130	17.3
Feral swine	53	1	1.9
Javelina*	13	0	0
Wild birds	383	0	0
Wild rodents/rabbits	776	0	0

\*Javelina samples collected during 2012 hunter harvest survey.

Table 2. *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* (STEC) frequency in wild birds by species, southwestern desert, 2013-2014.

Wild Bird (common name)	Sample size	Percent positive (%)		
		<i>Salmonella</i>	Non-O157 STEC	STEC O157
Black phoebe	1	0	0	0
Brown-headed cowbird	92	1 (1.1)	5 (5.4)	0
Chipping sparrow	1	0	0	0
Common grackle	1	0	0	0
Common ground dove	5	0	0	0
Common raven	4	1 (25.0)	0	0
Common yellowthroat	1	0	0	0
Eurasian collared dove	31	0	0	0
European starling	2	0	0	0
Gambel's quail	8	0	0	0
Great-tailed grackle	21	2 (9.5)	0	0
Green heron	1	0	0	0
House sparrow	5	0	0	0
Loggerhead shrike	3	0	0	0
Mourning dove	5	0	0	0
Pacific slope flycatcher	1	0	0	0
Red-winged blackbird	157	1 (0.6)	2 (1.3)	0
Vesper sparrow	4	0	0	0
Western kingbird	2	0	0	0
White-crowned sparrow	16	1 (6.1)	3 (18.8)	0
White-winged dove	3	0	0	0
Wilson's warbler	2	0	0	0
Yellow-headed blackbird	3	0	0	0
Yellow-rumped warbler	11	1 (9.1)	0	0
Unknown	3	1 (33.3)	0	0
Total	383	8 (2.1)	10 (2.6)	0

Table 3. *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* (STEC) frequency in wild rodents and rabbits by species, southwestern desert, 2013-2014.

Wild Rodents (common name)	Sample size	Number positive (%)		
		<i>Salmonella</i>	Non-O157 STEC	STEC O157
Botta's pocket gopher	1	1 (100.0)	0	0
Brush mouse	1	0	0	0
Cactus mouse	272	6(2.2)	0	0
Deer mouse	9	0	0	0
Desert cottontail rabbit	1	0	0	0
Desert kangaroo rat	1	0	0	0
Desert pocket mouse	264	7 (2.7)	1 (0.4)	0
Hispid cotton rat	15	0	0	0
House mouse	21	0	0	0
Long-tailed pocket mouse	18	0	0	0
Merriam's kangaroo rat	37	4 (10.8)	0	0
Rock pocket mouse	5	0	0	0
Round-tailed ground squirrel	12	2 (16.7)	0	0
Western harvest mouse	3	0	0	0
Western white-throated wood rat	114	4 (3.5)	0	0
Unknown	2	0	0	0
Total	776	24 (3.1)	1 (0.1)	0

Table 4. Seasonal pathogen of *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* (STEC) in cattle, wild bird, and wild rodent samples (seasonal peaks are highlighted in **bold**).

<i>Salmonella</i>	Spring	Summer	Fall	Winter
Cattle	2.5%	0.0%	<b>16.5%</b>	9.3%
Wild Birds	<b>3.7%</b>	0.0%	1.3%	1.7%
Wild Rodents	3.3%	2.0%	<b>3.4%</b>	3.3%
Non-O157	Spring	Summer	Fall	Winter
Cattle	32.0%	22.5%	<b>52.5%</b>	49.3%
Wild Birds	<b>5.9%</b>	0.0%	1.3%	0.0%
Wild Rodents	0.0%	0.7%	0.0%	0.0%
<i>E. coli</i> O157	Spring	Summer	Fall	Winter
Cattle	9.0%	<b>22.5%</b>	20.0%	18.0%
Wild Birds	0.0%	0.0%	0.0%	0.0%
Wild Rodents	0.0%	0.0%	0.0%	0.0%

Table 5. Birds fitted with Radio-transmitters by season.

Common Name	Spring	Summer	Fall	Winter	TOTAL
Brown-headed cowbird	6	0	1	4	11
Common raven	3	0	0	1	4
Eurasian collared dove	6	0	14	1	21
European starling	1	0	0	0	1
Red-winged blackbird	27	0	34	5	66
TOTAL	43	0	49	11	103



Table 6. O-serotype frequency among Shiga toxin-producing *Escherichia coli* (STEC) isolates obtained from cattle (N = 750) and wildlife (N = 1,225) samples in the southwestern desert (isolates belonging to the “top 7” clinically relevant serogroups are shown in **bold**).

O-antigen	Source					Total
	Cattle	Feral swine	Javelina	Wild bird	Wild rodent	
1	2	0	0	0	0	2
<b>103</b>	<b>3</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>4</b>
108	1	0	0	0	0	1
109	15	0	0	0	0	15
<b>111</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>2</b>
115	1	0	0	0	0	1
136	38	1	1	0	0	40
<b>145</b>	<b>5</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>5</b>
149	3	0	0	0	0	3
156	29	0	0	1	0	30
<b>157</b>	<b>130</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>131</b>
160	11	0	0	2	0	13
163	1	0	0	0	0	1
168	3	0	0	0	0	3
171	16	0	0	0	0	16
178	8	0	0	0	0	8
2	14	0	0	0	0	14
22	0	1	0	0	0	1
<b>26</b>	<b>3</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>4</b>
3	1	0	0	3	0	4
41	1	0	0	0	0	1
<b>45</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>
49	1	0	0	0	0	1
5	0	0	0	1	0	1
74	3	0	0	0	0	3
76	2	0	0	0	0	2
8	2	0	0	0	0	2
82	1	0	0	0	0	1
84	1	0	0	0	0	1
85	1	0	0	0	0	1
Untypable	15	0	0	0	0	15
pending	105	1	0	2	1	109
<b>Total</b>	<b>418</b>	<b>6</b>	<b>1</b>	<b>10</b>	<b>1</b>	<b>305</b>

Table 7. Virulence markers among Shiga toxin-producing *Escherichia coli* (STEC) serogroup O157 isolates obtained from cattle and wildlife samples in the southwestern desert.

Source	O157 STEC (%)	Number of samples	STEC O157 positive isolates	<i>fliC</i>	<i>stx1</i>	<i>stx1, eaeA</i>	<i>stx1, hlyA</i>	<i>stx1, eaeA, hlyA</i>	<i>stx2</i>	<i>stx2, eaeA</i>	<i>stx2, hlyA</i>	<i>stx2, eaeA, hlyA</i>	<i>stx1,2</i>	<i>stx1,2, eaeA</i>	<i>stx1,2, hlyA</i>	<i>stx1,2, eaeA, hlyA</i>
Cattle	17.3	750	130	117	0	0	0	0	2	3	0	27	0	6	0	92
Feral pig	1.9	54	1	1	0	0	0	0	0	0	0	1	0	0	0	0
Total	6.7	1962	131	118	0	0	0	0	2	3	0	28	0	6	0	92

Table 8. Virulence markers among non-O157 Shiga toxin-producing *Escherichia coli* (STEC) isolates obtained from cattle and wildlife samples in the southwestern desert.

Source	Non-O157 STEC (%)	Number of samples	Non-O157 STEC isolates	<i>fliC</i>	<i>stx1</i>	<i>stx1, eaeA</i>	<i>stx1, hlyA</i>	<i>stx1, eaeA, hlyA</i>	<i>stx2</i>	<i>stx2, eaeA</i>	<i>stx2, hlyA</i>	<i>stx2, eaeA, hlyA</i>	<i>stx1,2</i>	<i>stx1,2, eaeA</i>	<i>stx1,2, hlyA</i>	<i>stx1,2, eaeA, hlyA</i>
Cattle	38.4	750	288	3	16	1	52	20	135	1	10	43	1	1	3	5
Feral swine	7.5	53	4	0	0	0	0	4	0	0	0	0	0	0	0	0
Javelina	7.7	13	1	0	0	0	1	0	0	0	0	0	0	0	0	0
Wild bird	2.6	383	10	0	1	0	2	2	4	0	0	0	0	0	0	1
Wild rodent	0.1	775	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Total	15.5	1962	304	3	17	1	55	26	140	1	10	43	1	1	3	6

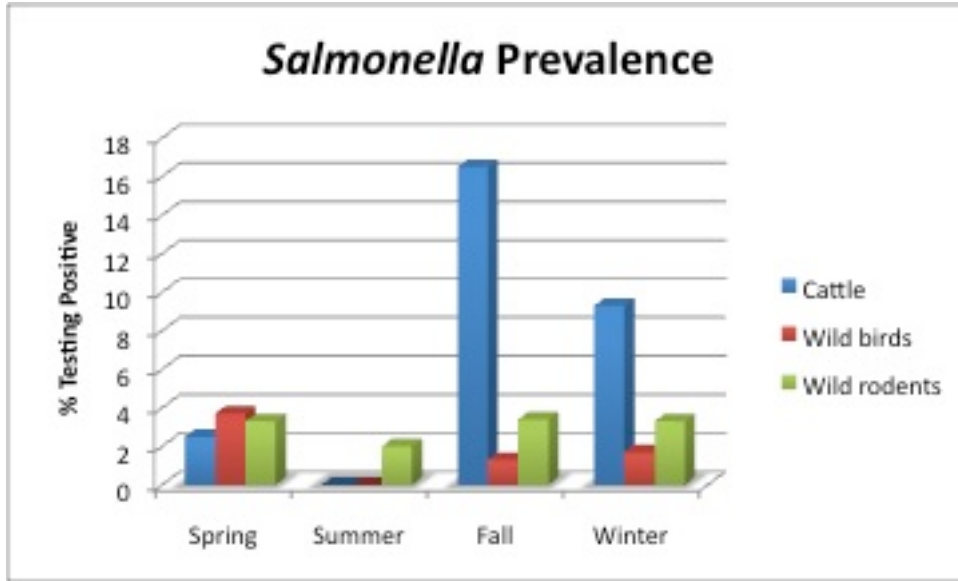


Figure 1. Recovery of *Salmonella* by season from cattle, wild bird, and wild rodent samples in the desert southwest, 2013-2014.

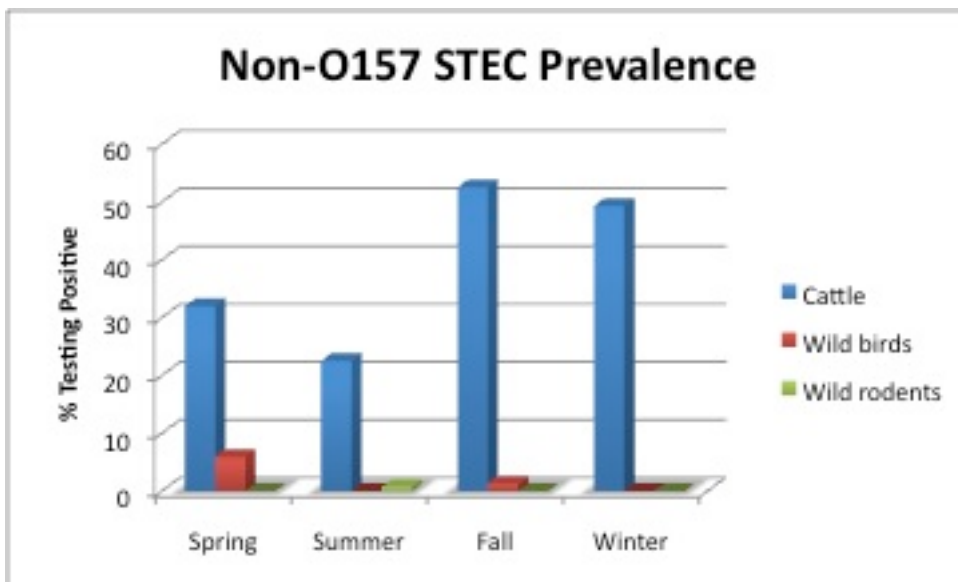


Figure 2. Recovery of non-O157 STEC by season from cattle, wild bird, and wild rodent samples in the desert southwest, 2013-2014.

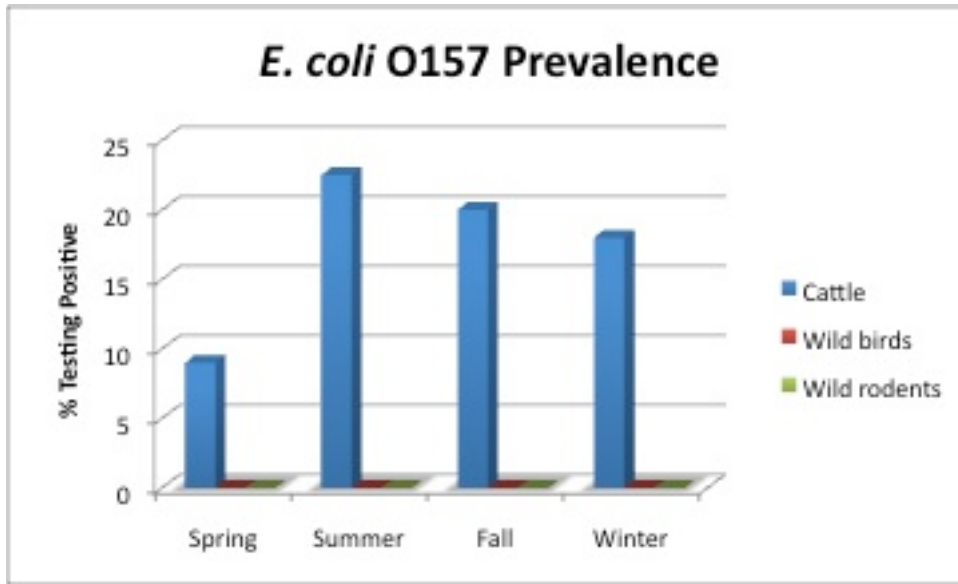


Figure 3. Recovery of STEC O157 by season from cattle, wild bird, and wild rodent samples in the desert southwest, 2013-2014.

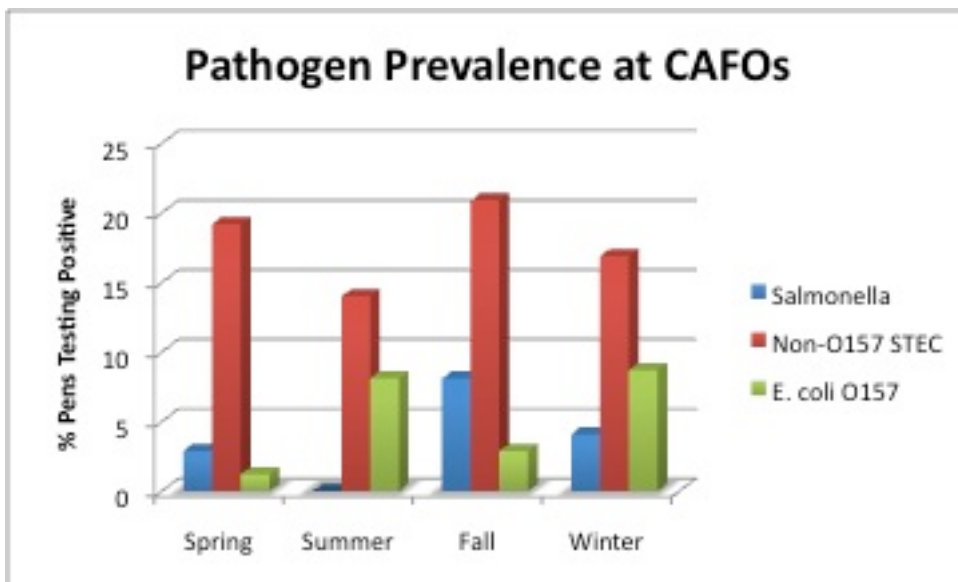


Figure 4. Pathogen prevalence by season in cattle feces at concentrated animal feeding operations (CAFOs), southwestern desert, 2013-2014.

Michele Jay-Russell, University of California, Davis

*Reducing the risk for transfer of zoonotic foodborne pathogens from domestic and wild animals to vegetable crops in the Southwest desert*



Figure 5. Use of radiotelemetry to track bird movement: (A) solar-powered receiver; (B) bird fitted with a transmitter; (C) "backpack" with transmitter on a red-winged blackbird.

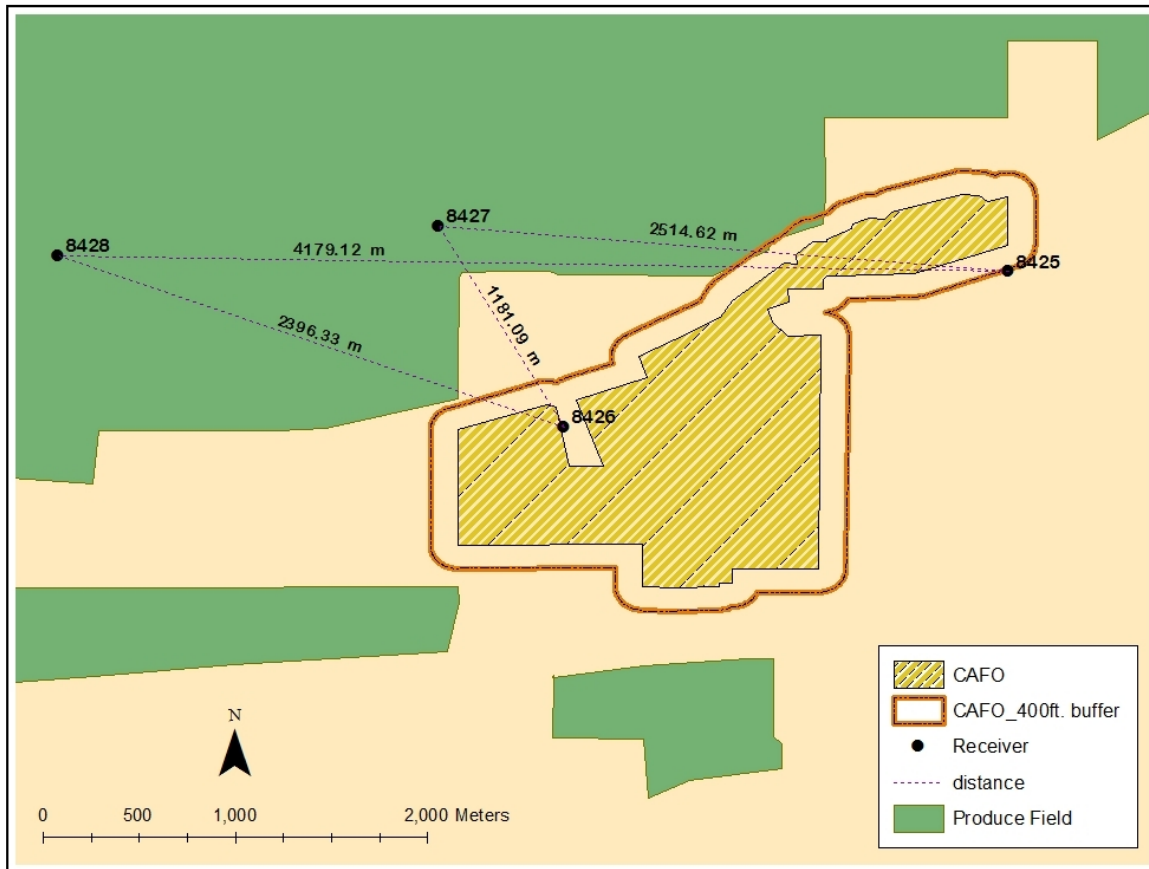


Figure 6. Map showing the CAFO (Arizona) in yellow, and including all the locations where livestock (cattle) are maintained in pens. The orange line surrounding the CAFO shows the 400ft buffer zone, the minimum area away from livestock that current guidelines recommend that fresh produce intended for human consumption not be planted. The green areas indicate fresh produce fields where the product is intended for human consumption. The black circles represent receivers that record bird movements. Receivers 8425 and 8426 are located at the CAFO, while 8427 and 8428 are located in fresh produce fields between 1 and 4 km away from the CAFO.

Michele Jay-Russell, University of California, Davis

*Reducing the risk for transfer of zoonotic foodborne pathogens from domestic and wild animals to vegetable crops in the Southwest desert*

**Suggestions to CPS (optional)**

None.



Michele Jay-Russell, University of California, Davis

*Reducing the risk for transfer of zoonotic foodborne pathogens from domestic and wild animals to vegetable crops in the Southwest desert*

## **Acknowledgments**

We thank the collaborators and contributors to this report including the growers who allowed access to their properties.

### University of California, Davis

Michele Jay-Russell, Principal Investigator

Paula Kahn-Rivadeneira (now at University of Arizona)

Peiman Aminabadi

Jessica Wong

Anna Zwieniecka

Derek Hilson

Alexis Hake

Trevor Suslow

Janneth Pinzon

### Arizona Game and Fish Department

Anne-Justice Allen, Co-Principal Investigator

Carrington Knox

Allie Hunter

### UCCE Desert Research and Extension Center

Fernando Miramontes

Gilberto Magallon

Khaled Bali

### University of Arizona, Yuma Agricultural Center

Kurt Nolte

Humberto Hernandez

### USDA Wildlife Services

David L. Bergman (Arizona)

Dallas R. Virchow (New Mexico)