CENTER for PRODUCE SAFETY

CPS 2010 RFP
FINAL PROJECT REPORT

Project Title

Developing buffer zone distances between sheep grazing operations and vegetable crops to maximize food safety

Project Period

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Objectives

1. Estimate the survival of *E. coli* O157:H7, commensal *E. coli*, and *Salmonella* spp. in sheep feces and soil where sheep graze (before and after irrigation).
2. Estimate the potential distance travelled by viable bacteria in aerosols generated by sheep grazing activity.
3. Based on pathogen survival and aerosol distance information, generate practical management guideline for growers to set scientifically valid “buffer zones” between livestock operations and crop production areas.

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Abstract

In the Imperial Valley of California, sheep are grazed on alfalfa fields during the winter months. This integration of crop and animal agriculture is beneficial to both entities, in terms of providing inexpensive forage for grazing sheep while providing income and organic matter (in the form of manures) for alfalfa producers. The potential for contamination of agricultural produce with pathogenic bacteria such as *E. coli* O157:H7 and *Salmonella* spp. have raised concerns related to the presence of livestock and wild animals in or near fresh market vegetable fields. We undertook this research project to develop data on prevalence of infection with these bacteria in sheep that can subsequently be used to accurately define "buffer zones" appropriate for grazing of sheep near production of leafy greens.

Over a period of 6 months, we collected and analyzed over 1,400 samples of feces and soil in alfalfa fields where sheep had grazed. *E. coli* O157:H7 was found in 1.8% of fecal samples and 0.4% of soil samples, while *Salmonella* were detected in 0.8% of fecal samples and 0.4% of soil samples. No significant associations between prevalence and management factors such as duration of grazing, irrigation events or source of sheep were detected. The overall mean coliform count from feces over the entire project was 1.05×10^7 CFU/g feces, while mean commensal bacteria count from soil was 3.5×10^3 CFU/g. Air sampling indicated that bacterial dispersion through the air was minimal. While a larger mean number of bacteria were collected at a distance of 2m from the field edge than from other distances, the difference was not statistically significantly different. It is important to note that wind speeds were always 5 mph or less during our collections.

Our results from this and previous studies indicate that sheep grazing on alfalfa in the Imperial Valley have a low prevalence of *E. coli* O157:H7 and *Salmonella* spp. in their feces and that these bacteria are rarely found in soil from fields with grazing sheep. Airborne dispersal of bacteria is possible, however the concentration of bacteria and distance traveled are both minimal. Based on our results, the current LGMA guideline of 30 ft between grazing lands/domestic animals and the edge of a crop is more than adequate to minimize any potential contamination of nearby crops.

Background

Ruminants play an important role in sustainable agricultural systems. Sheep are particularly useful in converting vast renewable resources from rangelands, pasture and crop residues into edible food.[1] Sheep producers in California are dependent on the use of inexpensive forage for grazing. In addition to the economic benefits associated with such practices, the manure produced by the sheep serves as an organic fertilizer that improves soil structure and contributes to plant nutrition. This grazing system in Imperial County involves intensive grazing for short time periods. Up to 1,500 head of sheep are typically turned into a 40 acre field. Once the forage is grazed close the sheep are moved to another field. If the next field is located nearby (within 2 to 3 miles), this is often accomplished by herding them along public roads. California ranks second in the nation for sheep production and contributes 50 million dollars to the California agricultural industry, producing over 3 million pounds of wool and 325,000 lambs annually.[2] The sheep industry relies heavily on the ability to graze crop, vineyard and orchard fields throughout California.

The Imperial Valley has long been recognized as the "winter salad bowl" for the United States. With over 100,000 acres of fresh market vegetable production with a farm gate value of one half billion

dollars and nationwide product distribution the industry has a tremendous impact on the local economy as well as nationwide food supply. Successful production of fresh market vegetables is dependent on the capacity of growers to rotate vegetable crops with crops that provide a suitable economic return while reducing pest pressure in the subsequent vegetable crop. Alfalfa is the standard rotation with vegetable crops in Imperial County.

The integration of crop and animal agriculture can however result in detrimental consequences. Contamination of agricultural produce with *Escherichia coli* O157:H7 has been documented through application of raw manure, use of contaminated irrigation water[3] and deposition of feces by livestock and wild animals.[4, 5] Recent outbreaks of human disease in California have been associated with consumption of raw spinach[4, 6] and lettuce.[7, 8]

Due to food safety concerns, over 99% of the volume of California leafy greens, including those grown in the Imperial Valley are produced and marketed under the California Leafy Green Products Handler Marketing Agreement (LGMA). The participating companies have committed themselves to sell products grown in compliance with the food safety practices accepted by the LGMA board. The board recognizes the need for further research to validate or adjust these guidelines based on scientific evidence. One area stated by LGMA as needing additional research relates to setback distances, or "buffer zones". There is a paucity of information related to appropriate combinations of time and distance between livestock operations and crop systems, particularly in terms of pathogen survival in animal feces, soil, and aerosols, as well as the pathogen movements through wind, water or flies. The LGMA suggests that a distance of 400 ft exist between a concentrated animal feeding operation and the edge of a crop and 30 ft for grazing lands/domestic animals, but recognize a lack of science on which to base this recommendation.[9]

While considerable attention has been paid to the prevalence of potential food-borne disease organisms in cattle, less is known about the epidemiology of *E. coli* O157:H7 in grazing sheep. Similar to cattle, prevalence of this organism in sheep varies considerably with levels as low as 0.2% being reported in some studies[10] and as high as 68% in others.[11] Given that there are approximately 650,000 sheep and lambs in California and as many as 150,000 in the Imperial Valley on a seasonal basis, knowledge of the ecology of important human pathogens associated with sheep is essential. Therefore, the primary objective of this research project was to develop data that can be used to accurately define "buffer zones" appropriate for grazing of sheep near production of leafy greens.

Research Methods

Fecal and soil samples were collected from alfalfa fields where bands of sheep, consisting of between 1,200 and 1,800 head of approximately 6-month old lambs from numerous locations throughout the Western United States, were grazing or had recently grazed. For each collection, 40 samples of fresh feces (minimum 10 g) and 40 samples of soil (minimum 10 g) were placed into individual containers and immediately placed on ice. Samples were shipped overnight by courier and processed within 24 hours of collection. Most bands of sheep were sampled once, however four groups were sampled twice and two groups were sampled three times.

Standard microbiological techniques were used to enumerate commensal *E. coli,* and to identify *E. coli* O157:H7 and *Salmonella* spp. Mean commensal *E. coli* and coliform bacteria concentration in feces and soil was determined by dispersing 1.0 g of feces or soil in 39 mL of phosphate buffered solution

(PBS) using a rotational mixer for 5 min. The feces/soil–PBS solution was then serially diluted (10^2, 10^3, 10^4, 10^5, 10^6). The *E. coli* concentration in diluted feces/soil–PBS solution was determined by direct membrane filtration and culturing onto CHROMagar EC (Chromagar Microbiology, Paris, France) at 44.58C for 24 h (American Public Health Association, 1989).

Fecal and soil samples were enriched for *Salmonella* spp. using US EPA Method 1682 (United States Environmental Protection Agency, 1998). *Escherichia coli* O157 samples were enriched in tryptic soy broth (TSB), exposed to an immunomagnetic separation step, and then cultured on cefixime potassium tellurite sorbitol MacConkey (CT SMAC) and Rainbow agar containing novobiocin and tellurite (NT Rainbow) as previously described.[8, 12] *E. coli* O157:H7 colonies identified were further analyzed by real-time PCR (RT-PCR) to detect presence of virulence genes. Pulsed-field gel electrophoresis was performed on *E. coli* O157:H7 isolates with the standard PulseNet procedure by using XbaI restriction enzyme.[12, 13]

Air samples were collected from the edge of the field where the sheep were grazing. Samples were collected in duplicate or triplicate at each collection distance, which consisted of 2, 5, 10, 20, 50, and 100 m from the field edge. A sample was also obtained from an upwind location to serve as a control. The prevailing wind direction was used to determine which side of the field was sampled. The Microbial Air Monitoring System (MAS) – 100Eco (Merck) was used to test levels of total bacteria. The MAS-100 aspirates air at the rate of 100 l air per minute, and after initial tests it was determined that a sampling time of 10 min was appropriate given the low concentrations of bacteria in the air. Specific agar (Chromocult) was used to enumerate colonies, which was converted to colony forming units per cubic meter of air.[14] Air samples were obtained the same time/day as the fecal/soil collections. Air samples were collected on five additional occasions as well.

Meteorological data (wind speed, temperature, relative humidity, rainfall) was recovered from the closest California Irrigation Management Information System (CIMIS) weather station on a daily basis.[15]

Research Results

Sheep grazing is seasonal in the Imperial Valley. Typically, bands of 1,200 to 1,800 sheep arrive from throughout the western United States in October and rotate between 40 acre fields of alfalfa or Bermuda grass until approximately mid- to late-March when they are sent to a feedlot or directly to slaughter. Market prices for sheep, alfalfa, and other factors (such as transport cost) affect the utilization of this grazing system. For many different reasons, the number of sheep brought into the Imperial Valley has decreased significantly over the past several years. Historically, as many as 300,000 sheep would be present; however, in the fall of 2011, approximately 120,000 sheep were brought for grazing through the winter. There are only about 4 separate producers in Imperial Valley who currently utilize this grazing system. With alfalfa prices at record high levels, it is anticipated that even fewer producers will bring even fewer sheep to be grazed here in the future.

An informal survey was administered to several sheep ranchers and their responses indicated that historically (15 to 20 years ago) their sheep may have grazed on vegetable stubble (primarily broccoli fields); however this practice no longer occurs. A “typical” crop rotation for the Imperial Valley will consist of alfalfa for 4 years, followed by wheat or vegetables for 2-3 years, and then alfalfa is resown. Of interest, the vast majority of sheep are moved between fields by driving them along a road or irrigation ditch (versus moving them in trailers or trucks).

Samples were collected from January 2011 to March 2011 and again from October 2011 to December 2011. Total precipitation during these two time periods was 1.84 inches (2011 total precipitation was 2.04 inches), average air temperature was 56.5^{0}F and average wind speed was 4.2 mph. A total of 1440 individual fecal and soil samples were collected throughout the project. Of the 720 fecal samples, 13 (1.8%) were found to be positive for *E. coli* O157:H7, and of the 720 soil samples, 3 (0.4%) were positive for *E. coli* O157:H7. The highest prevalence in feces at any one sample collection was 10% (4 positive out of 40 samples). *E. coli* O157:H7 positive fecal samples were obtained at 7 of 18 sample collections and *E. coli* O157:H7 positive soil samples were obtained at 2 of 18 sample collections. There were no statistically significant differences in the proportion of positive samples on any of the collection dates. No significant associations between prevalence and management factors such as duration of grazing, irrigation events or source of sheep were detected. There was also no association between duration of sheep grazing and presence of bacteria in the soil. The data is shown in Table 1. Pulsed-field gel electrophoresis was performed on several of the *E. coli* O157:H7 isolates recovered (Figure 1). In general, isolates from the same date and same group of sheep shared a PFGE pattern, while other groups of sheep sampled on different dates had unique patterns.

Salmonella spp. was detected in 6 (0.8%) fecal samples and 3 soil samples (0.4%). All positive soil samples were obtained on the same sampling date, while fecal positive samples were obtained from 3 sampling dates. Interestingly, a significant precipitation event (over 1 inch within a 2-day period) occurred a few days before the *Salmonella* positive soil samples were collected. The data is shown in Table 1.

The mean commensal *E. coli* and coliform bacteria concentration in feces and soil were also measured. These results are shown in the figures below. The overall mean coliform count from feces over the entire project was 1.05×10^7 CFU/g feces, while mean commensal bacteria count from soil was 3.5×10^3 CFU/g soil. Finding coliform bacteria in feces and soil reassured us that shipping the samples via overnight courier did not result in significant reduction of bacterial counts.

Figure 1. Fecal *E. coli* bacterial counts from sheep feces collected in the Imperial Valley, California, in 2011 (expressed as log (10) Colony forming units/g feces).

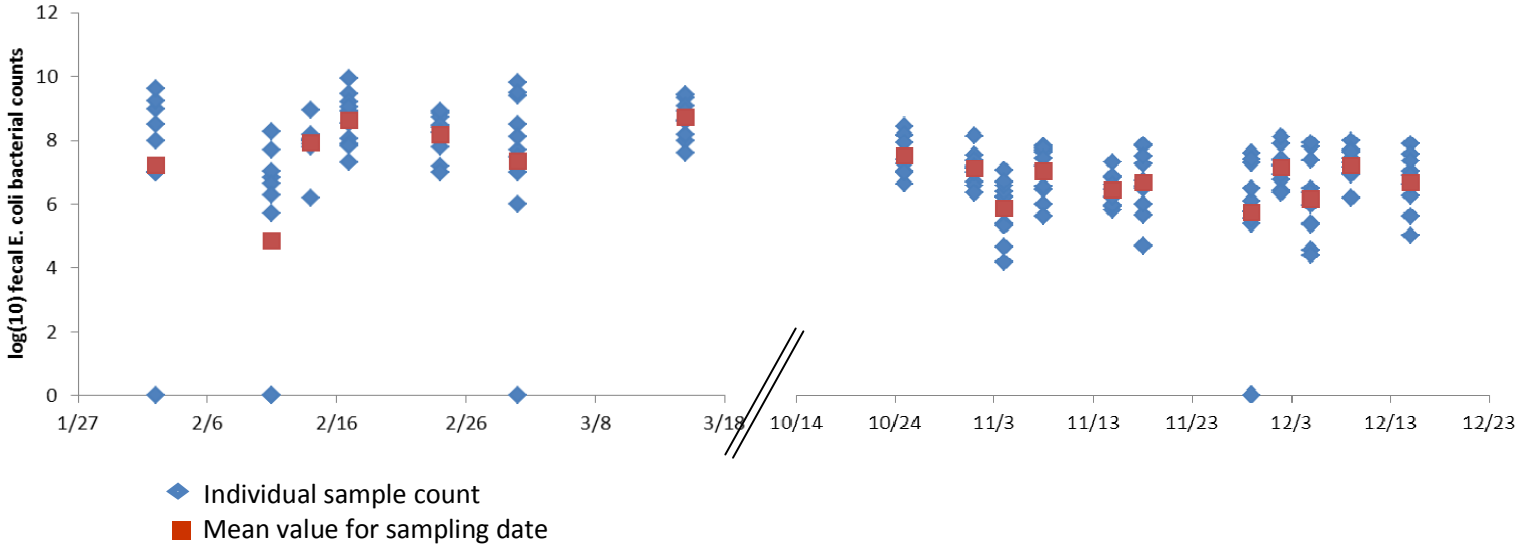
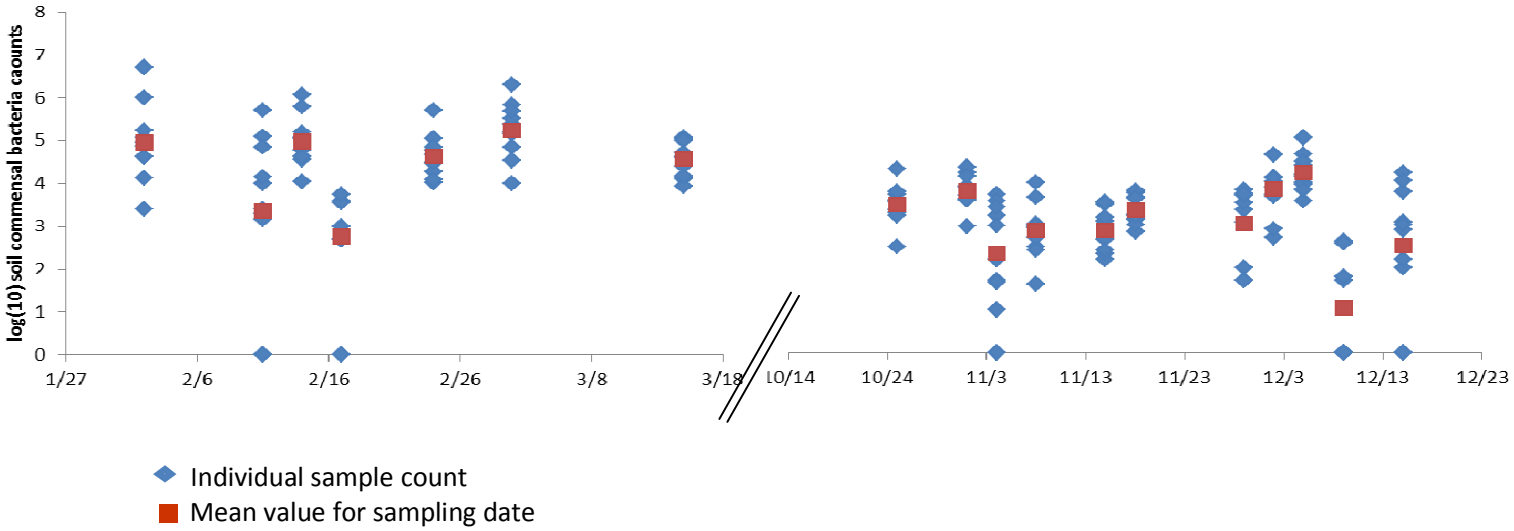
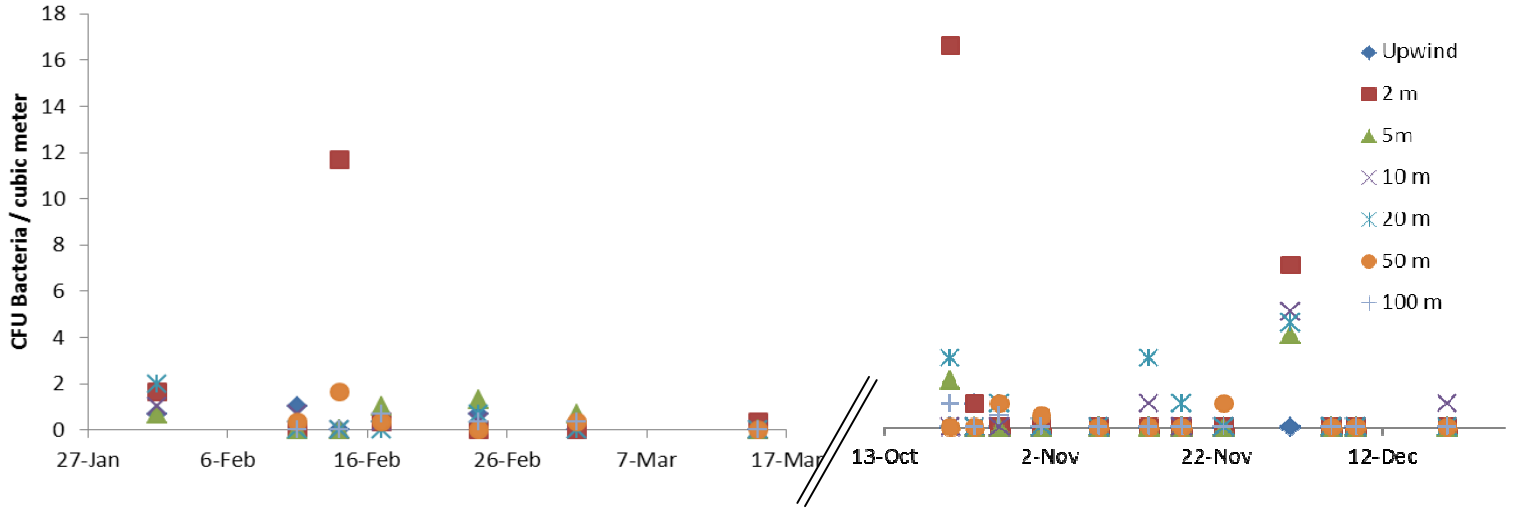


Figure 2. Commensal bacterial counts from soil collected in fields with grazing sheep in the Imperial Valley, California, in 2011 (expressed as log (10) Colony forming units/g soil).



Air sampling revealed that few bacteria were being dispersed through the air. The maximum number of colony forming units per cubic meter of air was 16.5 from a sample obtained on October 21. The mean number of CFU/m^3 was greatest at a distance of 2m, however a one-way analysis of variance demonstrated that there was no statistically significant difference at any distance measured, nor was there significant correlation between distance and bacterial count. Using linear regression it was determined that there were no significant correlations between fecal/soil *E. coli* counts and aerosol bacterial counts at any of the distances measured. The raw data obtained from the air sampling is presented in Table 2.

Figure 1: Mean number of colony forming units of bacteria per cubic meter of air from samples obtained at various distances from the edge of alfalfa fields with sheep present and grazing.



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Outcomes and Accomplishments

This project demonstrated that the prevalence of the important human pathogens, *E. coli* O157:H7 and *Salmonella* in feces from sheep grazing alfalfa in the Imperial Valley is very low. Less than 2.5% of all fecal samples collected contained one of these pathogens. An even smaller proportion of soil samples (0.8%) were found to harbor one of the bacteria of interest. The positive soil samples were observed to be associated with recent (within 5-7 days) rainfall events. This observation requires further study, as positive soil samples were obtained at only 3 sample collections.

Dispersal of bacteria through air can occur, but the concentration and distance traveled are minimal. A "buffer zone" of 30 ft between grazing livestock and crops, as suggested in the LGMA guidelines, should provide sufficient protection from potential contamination.

The outcomes of the project match very closely with the original objectives. Many of the analysis returned non-significant results, such as the relationship between management factors and prevalence of fecal pathogens. We believe that for many of our analyses, this was related to the very low prevalence of pathogens detected. While larger sample sizes may have resulted in some significant findings, we are somewhat limited by laboratory capacity.

An unexpected "outcome" was that the number of sheep arriving into the Imperial Valley on an annual basis has fallen dramatically in recent years. Economic pressures (primarily an increase in alfalfa price and therefore an increase in grazing fees to sheep producers) are driving much of this change. Producers we visited with believe that this trend will continue and that sheep grazing in the Valley has a high likelihood of disappearing within the next several years.

The completion of this project required collaborative efforts from many individuals and groups. The California Woolgrowers Association provided us with sheep-producer contacts in the Imperial Valley. The sheep producers we worked with were amazingly cooperative. They allowed us free access to their flocks and answered all our questions. Without their assistance, the project could not have occurred. University of California Cooperative Extension, Holtville, provided us with laboratory space to conduct the air sampling portion of the project. We were also fortunate to be able to enlist a staff member from a researcher's laboratory to assist us with our sampling effort. Our original collaborator from UCCE (Dr. Henderson) left Holtville after the spring sampling, so we are very grateful to the Holtville center for providing us with support. Finally, the staff in the Atwill Water and Foodborne Zoonotic Disease Laboratory must be commended for the very rapid and complete analysis of all the samples collected.

Summary of Findings and Recommendations

Integrated livestock and crop operations are beneficial to producers of both products. Crop residues are an important source of food for livestock, however domestic and wild animals represent a potential source of foodborne pathogens. Recent outbreaks of human infection with *E. coli* O157:H7 and other bacteria linked to consumption of California produce have raised concerns that sheep and other ruminants may elevate levels of pathogens within the soil, which have the potential of being transmitted to produce fields via aerosols. The California Leafy Green Products Handler Marketing Agreement (LGMA) of January 2012 lists sheep as one of five mammalian species as "Animals of Significant Risk" and any intrusion by such animals requires a detailed food safety assessment prior to harvest. "Buffer

zones" between the crop production fields and livestock operations are important in order to prevent the potential transmission of pathogens from animals to crops.

We detected *E. coli* O157:H7 in 1.8% of fecal samples and 0.4% of soil samples. *Salmonella* spp. was found in 0.8% of fecal samples and 0.4% of soil samples. These results indicate that fresh sheep feces are low (but not zero) risk products for leafy green producers. Soil where sheep have been actively grazing is even lower (but still not zero) risk. For these reasons, the LGMA recommendation of a food safety assessment following intrusion by sheep is warranted. The PFGE patterns indicate that unique strains of *E. coli* O157:H7 exist in this population, and this information can be useful if investigations are necessary.

Airborne transmission of bacteria was also assessed in this project. The greatest number of bacteria was recovered at a distance of 2m from the edge of a field with sheep grazing. This difference was not significant, however. Given the low prevalence of pathogenic bacteria in either feces or soil, we were not able to detect these pathogens in air samples. Future studies that compared the genotypes of bacteria recovered by air sampling with bacteria from the sheep would be worthwhile. Based on our findings, we believe that the LGMA recommended buffer distance of 30 ft (9m) between grazing livestock and crops is justified, and will provide a more than adequate distance to ensure protection from potential contamination by grazing sheep.

APPENDICES

Publications and Presentations

There have been no publications or presentations resulting from this project thus far. We expect to submit a manuscript summarizing the past two CPS-funded grants in the near future (within 4 - 6 months).

Budget Summary

Grant funds were used for salary ($37,257), supplies ($52,417), travel ($1,867), and benefits ($11,928). We were fortunate to have an incredibly efficient staff to process the samples collected. This resulted in far less expense for salaries (and therefore benefits) than we had initially budgeted. Also, we did not locate a graduate student to work on the project, therefore tuition and fees were not required. Similarly with travel – we had anticipated the need for the investigators to travel to the study site more frequently, however the personnel was highly adept at meeting all our needs, in terms of sampling. Supplies were over drafted by approximately $3,400. There is a balance of over $58,000 remaining.

Tables and Figures

Table 1: Prevalence of *E. coli* O157:H7 and *Salmonella* spp. in sheep feces and in soil from alfalfa fields with grazing sheep present from the Imperial Valley of California, 2011. Forty fecal and 40 soil samples were obtained for each collection.

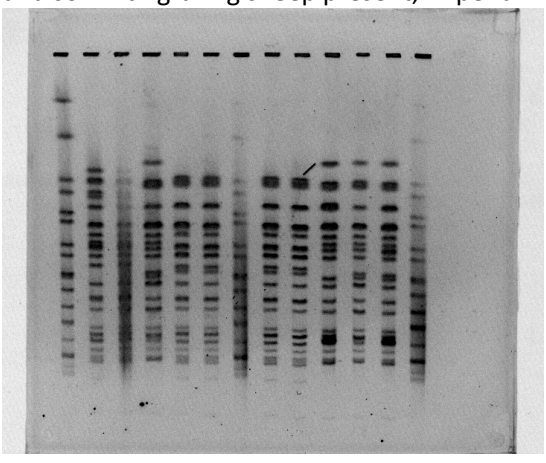
Date	Fecal *E. coli* O157:H7	Fecal Salmonella	Soil *E. coli* O157:H7	Soil Salmonella
Feb 2	0	0.025	0	0
Feb 11	0	0	0	0
Feb 14	0	0	0	0
Feb 17	0	0	0	0
Feb 24	0	0	0	0.075
Mar 2	0	0.075	0	0
Mar 15	0.025	0	0	0
Oct 25	0.025	0.025	0.05	0
Nov 1	0	0	0	0
Nov 4	0	0	0	0
Nov 8	0.05	0	0	0
Nov 15	0	0	0.025	0
Nov 18	0	0	0	0
Nov 29	0.05	0	0	0
Dec 2	0.1	0	0	0
Dec 5	0.025	0	0	0
Dec 9	0.05	0	0	0
Dec 15	0	0.025	0	0

Table 2: Mean number of colony forming units of bacteria per cubic meter of air at specific distances downwind from sheep grazing in alfalfa fields, Imperial Valley, California, 2011.

Date	Upwind	2 m	5m	10 m	20 m	50 m	100 m
1-Feb	0.67	1.67	0.67	1	2	NA	NA
11-Feb	1	0	0	0.67	0	0.33	0
14-Feb	0	11.67	0	0	0	1.67	0
17-Feb	0.33	0.33	1	0.33	0	0.33	0.67
24-Feb	0.67	0	1.33	0.33	0.67	0	0.33
2-Mar	0	0	0.67	0	0	0.33	0.33
15-Mar	0	0.33	0	0	0	0	0
21-Oct	0	16.50	2	0	3	0	1
24-Oct	1	1	0	0	0	0	0
27-Oct	0	0	0	0	1	1	0.50
1-Nov	0	0	0	0	0	0.50	0
8-Nov	0	0	0	0	0	0	0
14-Nov	0	0	0	1	3	0	0
18-Nov	0	0	0	0	1	0	0
23-Nov	0	0	0	0	0	1	0
1-Dec	0	7	4	5	4.5	NA	NA
6-Dec	0	0	0	0	0	0	0
9-Dec	0	0	0	0	0	0	0
20-Dec	0	0	0	1	0	0	0
Mean	0.19	2.03	0.51	0.49	0.80	0.30	0.17

NA = samples not available due to proximity to other structures.

Figure 1. Pulsed-field gel electrophoresis of some *E. coli* O157:H7 isolates recovered from sheep feces and soil with grazing sheep present, Imperial Valley, California, 2011.



Lane 1, 7, and 13 are control isolates. Lane 2 and 3 are from 10/25/11, lane 4 from 11/8/11, lanes 5, 6, 8, and 9 from 12/2/11, lane 10 from 12/5/11, and lanes 11 and 12 from 12/9/11.

Suggestions to CPS

No suggestions.

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