UC DAVIS
CENTER FOR PRODUCE SAFETY

CPS - CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

FINAL PROJECT REPORT, DUE APRIL 30, 2010

Project Title
Food safety risks associated with sheep grazing in vegetable stubble fields

Project Period
April 1, 2009 through March 31, 2010

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Objectives
Objective 1: Estimate the prevalence of fecal shedding of *E. coli* O157:H7 and *Salmonella* spp. by sheep grazing in different crop systems. We will also measure the intensity of fecal shedding of commensal *E. coli* to support objective (3).

Objective 2: Determine if rotational grazing between crop systems of differing forage quality and energy content alters the prevalence of fecal shedding of *E. coli* O157:H7 and *Salmonella* spp. by sheep.

Objective 3: Measure the rate of inactivation of *E. coli* O157:H7 and *Salmonella* spp. as a function of such parameters as time, tillage practice, irrigation, ambient temperature, etc., and compare these estimates to the fate of commensal *E. coli*.

Bruce Hoar, UC Davis

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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 particularly 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 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 move to another field, often by herding them along public roads. Likewise, 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. Of the approximately half-million acres under irrigation in Imperial County, almost half is seeded to alfalfa, which had a gross value in 2007 of $113,853,000.[2]

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 in California have been associated with consumption of spinach[4, 6] and lettuce.[7, 8]

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. Due to food safety concerns, over 99% of the volume of California leafy greens 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. In addition, certain large customers of leafy green products have imposed cumbersome and exhaustive requirements for production of leafy greens purchased by their company. One such customer has announced they would not purchase leafy greens from the largest vegetable production area in the Imperial Valley due to sheep grazing. In order to assess the validity of these concerns and to develop science-based recommendations regarding sheep grazing and food safety, several key questions related to the prevalence and intensity of *E. coli* O157:H7 and *Salmonella* spp. shedding, pathogen survival and inactivation after being deposited onto the soil surface or paved/graveled/dirt roads, and effect of crop system being grazed on pathogen prevalence must be addressed.

Objectives:

To address some of the concerns of both the produce and sheep industries, the following objectives were outlined:

1. Estimate the prevalence of fecal shedding of *E. coli* O157:H7 and *Salmonella* spp. by sheep grazing in different crop systems. We will also measure the intensity of fecal shedding of commensal *E. coli* to support objective (3).

2. Determine if rotational grazing between crop systems of differing forage quality and energy content alters the prevalence of fecal shedding of *E. coli* O157:H7 and *Salmonella* spp. by sheep.

3. Measure the rate of inactivation of *E. coli* O157:H7 and *Salmonella* spp. as a function of such parameters as time, tillage practice, irrigation, ambient temperature, etc, and compare these estimates to the fate of commensal *E. coli*.

Materials and Methods:

It was apparent from the outset that sampling individual animals when they were present in "bands" of approximately 1,500 head would be a poor representation of risk, therefore we concluded that the "band" would be the experimental unit and pooled fecal samples would more accurately represent the collective potential for pathogen shedding. Two to 8 samples per band, with approximately 50 to 150 individual animal fecal samples per pooled sample were collected on a weekly basis (some weeks were missed due to rainfall prior to sampling). Standard microbiological techniques were used to recover *E. coli* O157:H7, *Salmonella* sp., and commensal *E. coli.*

Outcomes and accomplishments:

While we initially envisioned sampling from 5 groups of sheep, our change to pooled sampling allowed us to increase the number of groups sampled. Therefore, a total of 19 unique "bands" of sheep were sampled between 11/12/09 and 03/11/10 on 14 separate sampling dates. Sheep are generally not present in the Imperial Valley at other times of the year. We began the trial by sampling from four bands repeatedly (7 to 9 times). After our initial culture results were available, we agreed that more information would be gained by sampling from a greater number of bands, therefore 15 other bands were identified and each sampled once. We estimate that approximately 28,000 sheep were in the population from which we obtained samples.

Nine bands had at least one positive culture for *Salmonella* sp. One band was positive on two consecutive sampling dates while on different alfalfa fields, while the other 8 positive bands were positive on one sampling date each. For the majority of the isolates, serotyping has not yet been possible (8/12) and one isolate is still pending. We will submit these isolates for further analysis (DNA sequencing) which may determine the serotype; otherwise it is possible that we have isolated a new strain(s). The three isolates we have confirmed are S. 4, 5:i:-, S.

Thompson, and S. Enteritidis. Samples were found positive for *Salmonella* on 6/14 sampling dates

No fecal samples were positive for *E. coli* O157:H7. We were concerned that this might be associated with pooling of samples resulting in lower detection sensitivity, therefore we performed a trial where we "spiked" fecal samples with known numbers of *E. coli* O157:H7 and then used our culture methods to determine at what level we could recover the organism. We were encouraged to learn that when 5 bacteria were placed into 10 g of sheep feces, our method would call the sample "positive" in greater than 90% of replicates.

Commensal *E. coli* was present at varying levels, ranging from 5×10^5 cfu/g feces to $> 1 \times 10^{10}$ cfu/g feces. These results are shown in the appendix as Table 1. We were encouraged by these results, as this indicated to us that shipping the samples via overnight courier did not result in significant reduction of bacterial counts.

We were unable to address Objective 2 for two reasons. First, the prevalence of shedding for *E. coli* O157:H7 and *Salmonella* spp. was too low for us to analyze any possible risk factors / associations that may have been present. Also, unlike previous winters, sheep were grazed entirely on alfalfa for the entire winter (with the exception of one week of sampling where Bermuda grass was grazed). Since there was no variability in the crop system used, we could not determine whether forage quality or energy content would affect shedding of either pathogens or commensal *E. coli*.

For objective 3, we were restricted to summarizing results for commensal *E. coli*, since pathogens were rare. Our studies indicated that commensal *E. coli* could be recovered from soil for less than 5 days under field conditions. Since all the grazing was of alfalfa, no tilling was performed.

We were extremely fortunate to have Mark Trent as a Co-PI on our grant. He was the primary resource involved in gathering and shipping the samples. His tireless work was invaluable to completion of the project. Sample collection would involve walking through alfalfa fields and collecting fecal samples deposited on the ground. Each field collection would take about 45 minutes to complete, therefore at least one morning per week was spent collecting samples. Mark would Fed-Ex everything to Davis, where the sample would be split between two labs, where bacterial isolation was performed.

Some difficulties arose during the project period. As stated above, prevalence of pathogens was very low (both a positive and negative outcome). This meant that certain data analyses could not be performed, such as risks for shedding. Also, we expected some variation in crops utilized by the sheep for grazing, however, alfalfa was almost exclusively grazed. An unusually "wet" winter also affected our ability to collect samples. To minimize damage to the alfalfa fields, sheep are confined to small 1-2 acre holding pens during rainfall events. If this occurred when we had planned on sampling, we were asked to forgo collection during that week.

Planning and carrying out the project involved tremendous cooperation from the sheep producers. California Wool Growers Association has a very strong membership, therefore identifying collaborators was straightforward. The producers were more than willing to allow us to collect samples, as they are very motivated to maintain access to crop stubble for grazing. The produce growers were also very interested in our study, which enabled us to implement the project with relative ease.

Funding for the project was adequate. We were able to fully implement the project, with minor exceptions. The majority of the budget was used for salary for laboratory support and materials used to isolate bacteria. Given that fewer pathogens were isolated than expected, expense for this category was less than expected. Reduced travel costs were offset by a larger than anticipated bill for Fed-Ex shipping.

Results from this project were presented at the California Leafy Greens Research Board Annual Meeting, March 16, 2010 at Harris Ranch Inn near Coalinga, CA (Powerpoint file attached). A manuscript for submission to a scientific journal is currently in preparation.

Based on the current study as well as previous studies in our lab, we conclude that the prevalence and intensity of shedding of both *E. coli* O157:H7 and *Salmonella* sp. in sheep in the Imperial Valley is apparently low. Further studies are warranted to follow-up on these findings.

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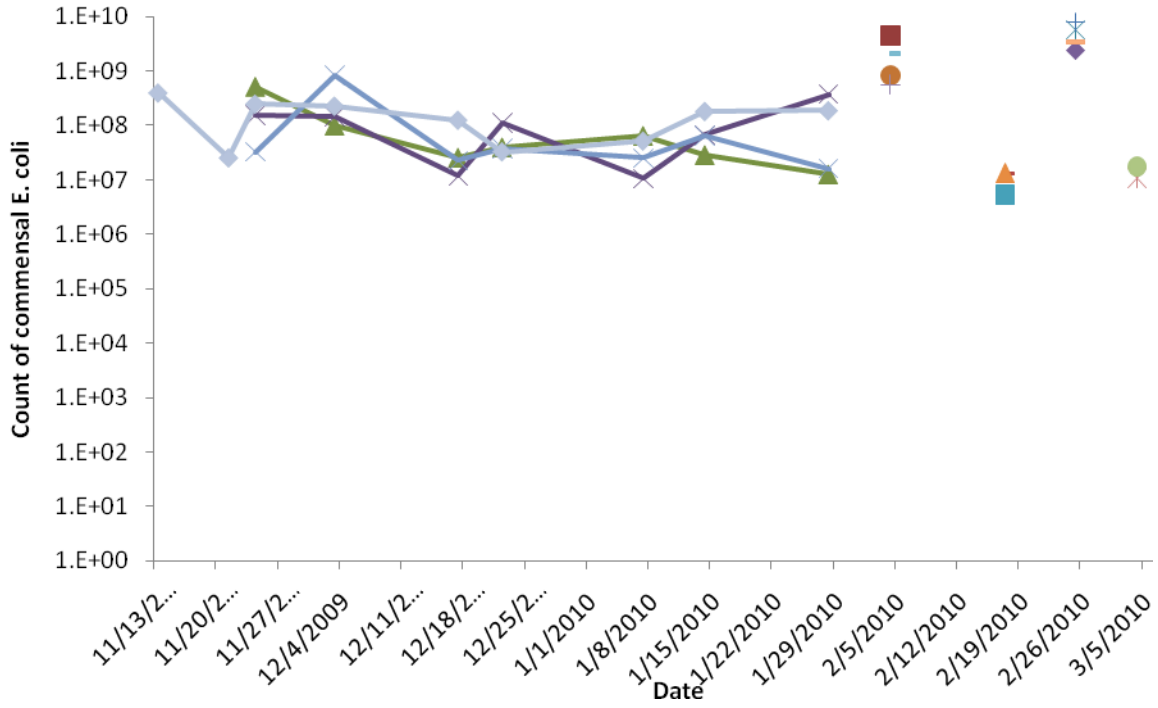
Appendix

Figure 1. Counts of commensal *Escherichia coli* recovered from sheep feces, Imperial Valley, CA during the period of 11/13/2009 – 3/5/2010.



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Unifying the voice of the **California Sheep Industry** since 1860

UC CE University of California Cooperative Extension

Presentation outline

- What other people have found in cattle and sheep
- What we have found in sheep in previous studies
- What we have found in Imperial Valley sheep
- Conclusions
- Questions

E. coli O157:H7 in cattle

- Prevalence ranges from:
  - 0.3 to 19.7% in feedlot
  - 0.7 to 27.3% on pasture
  - 0.9 to 6.9% on range
  - 0.2 to 27.8% at slaughter
- Prevalence influenced by:
  - Animal age, breed, sex, stage of production
  - Environment and management factors
  - Diet: DFM, DG (wet or dry), other feed additives such as ionophores, beta-agonists, etc

Controlling *E. coli* O157:H7 in cattle

- Slaughter house controls
- Direct-fed microbials, dietary management
- Water trough / feed bunk management
- Control access to flowing water
- Vaccination
  - Two vaccines with conditional licenses now available

E. coli O157:H7 in sheep

- Far fewer studies!
- No direct evidence of human illness from consumption of sheep meat, but 2 environmental outbreaks linked to sheep have occurred in Scotland

E. coli O157:H7 in sheep

- Prevalence ranges from:
  - 9.0% in feedlot feces, 18% in wool (Texas)
  - 7% of adults at slaughter (Italy)
  - 0% to 60% on pasture
  - Root crops in Scotland: 5.8% of animals positive from 43.8% (6/14) of farms, shedding at low concentrations of bacteria
- Prevalence influenced by ?
  - Diet? Season? Day length?

Prevalence of *E. coli* O157:H7 in California sheep

- 3 participating groups of sheep
  - "A" ewe flock of ~300 head (irrigated pastures)
  - "B" ewe flock of ~800 head (native rangeland)
  - "C" feedlot with up to 20,000 head capacity

    (primarily grain diet for up to 60 days)

Prevalence of *E. coli* O157:H7 in California sheep

- 40 fresh fecal samples collected per r

  from each group for 12 months

- Recovery of *E. coli* O157:H7

  10g feces
  → enrichment broth (tryptic soy broth)
  → immunomagnetic separation (Dynal®)
  → 2 selective media (CT- SMAC, Rainbow agar)
  → immunoblotting
  → PCR (*rfb*E, *stx*1, *stx*2, *eae*, *hly, fli*C)

MLVA (Multiple-locus variable-number tandem repeat analysis)

- Determines the difference in the number of tandem repeats (TRs) in multiple loci using PCR amplification
- 11 loci amplified in 3 multiplex PCR reactions
  - Vhec1, Vhec2, Vhec3, Vhec4, Vhec5, Vhec6, Vhec7,O157-17, O157-19, O157-25, O157-37
- PCR product sequenced, size of fragments determined with GeneMapper software, converted to number of TRs

Results: Crude prevalence

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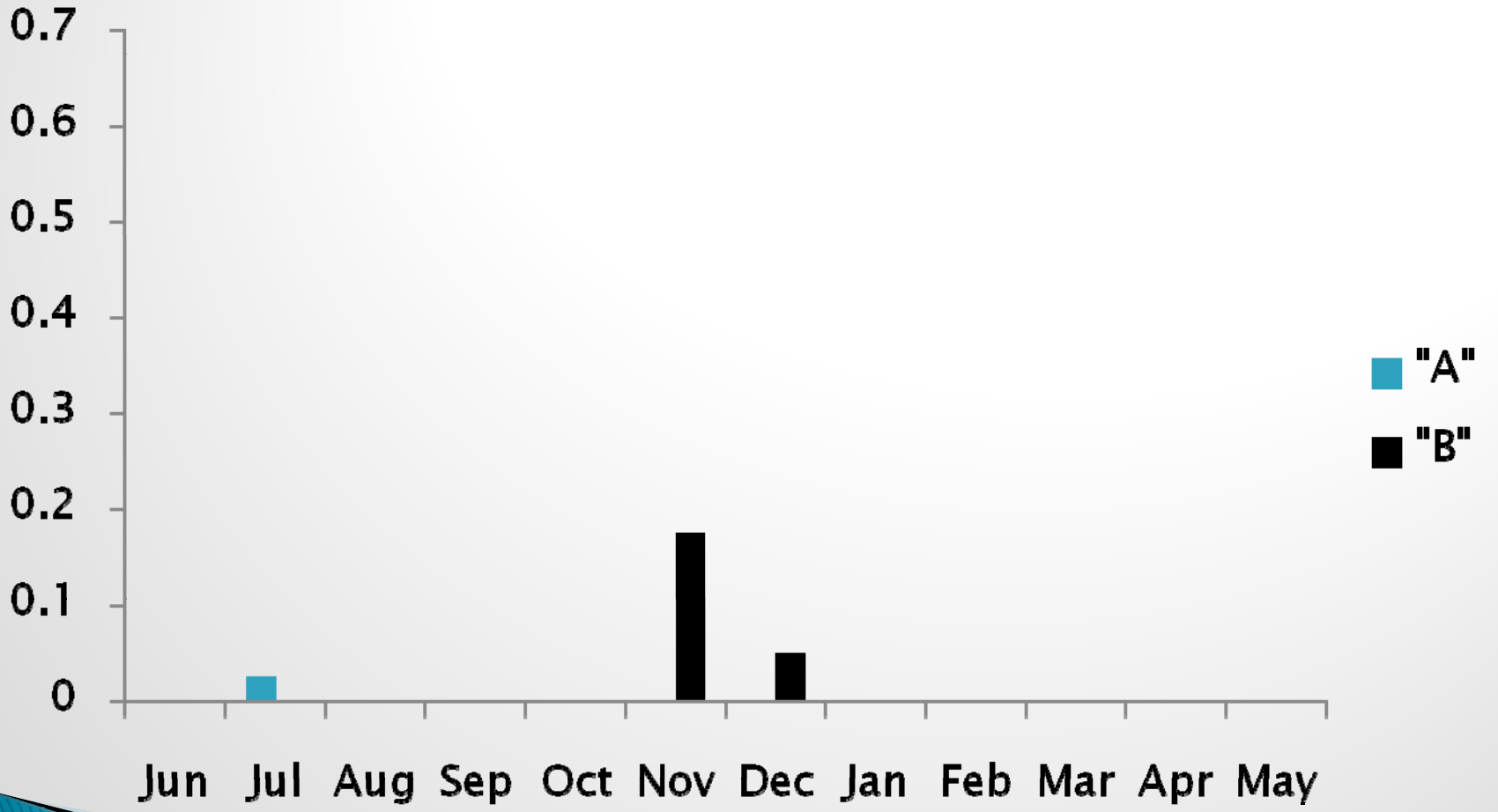

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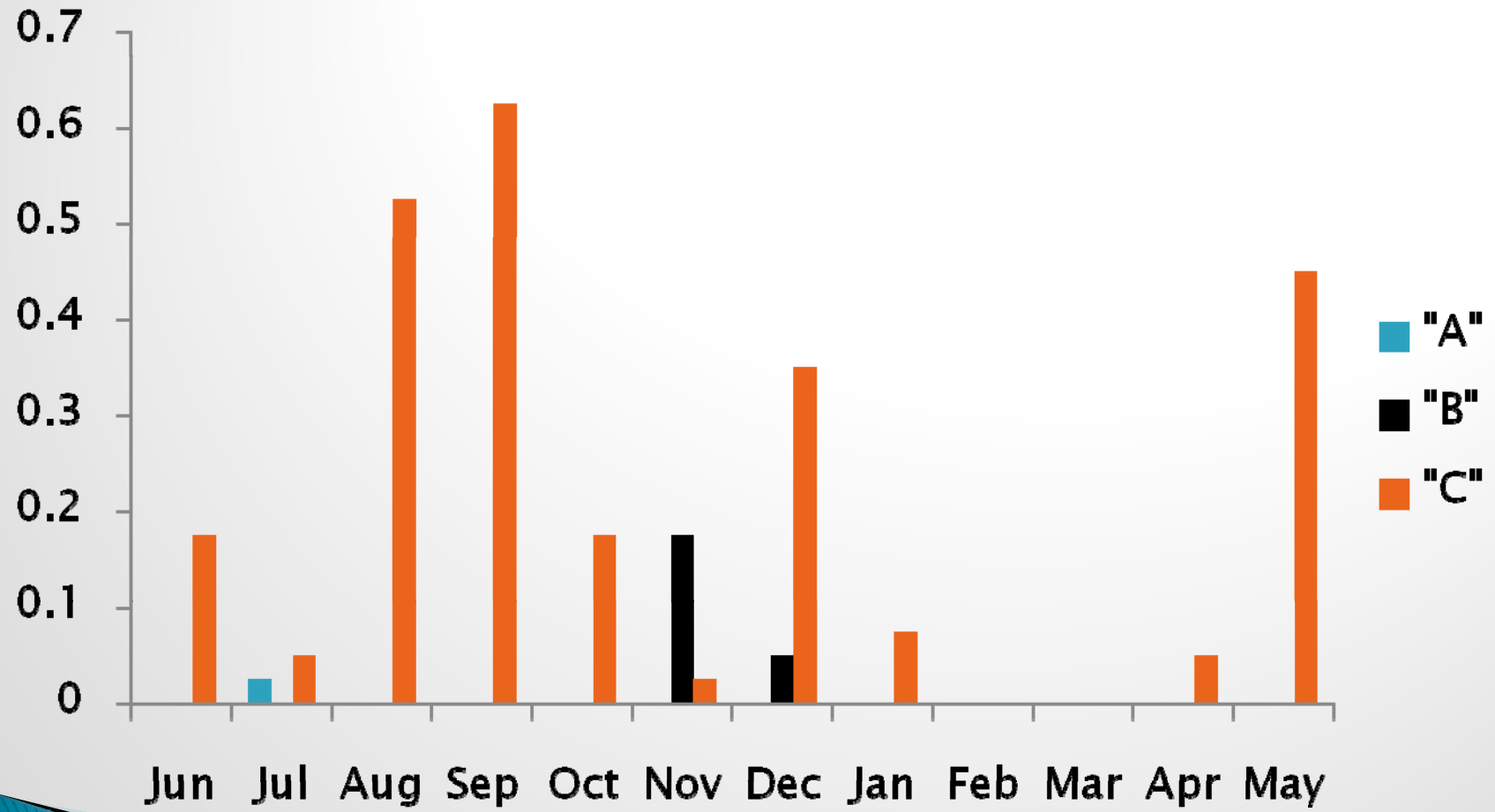

No sheep in feedlot in Feb

Results: MLVA Strain types

- Awaiting final analysis from graduate student
- Flock “A” sample likely contaminant
- Flock “B” – all isolates identical
- Feedlot “C” – only 4 or 5 distinguishable types

Conclusions (preliminary)

- Prevalence in sheep on range very low
- Potential seasonal effect?
  - Flock “B” from Mendocino County
- Prevalence much higher in feedlot setting
  - Surprisingly low variability possibly indicating that *E. coli* O157:H7 present in the feedlot environment, sheep infected after arrival

Imperial Valley Sheep production

- Of the approximately half-million acres under irrigation in Imperial County, almost half is seeded to alfalfa which had a gross value in 2007 of $113,853,000.
- Each year 200,000 to 300,000 lambs graze winter alfalfa in Imperial County, the largest concentration of sheep in the nation during that period. Smaller numbers of sheep in last few years, approximately 100,000 this past winter

Food safety risks associated with sheep grazing in stubble fields

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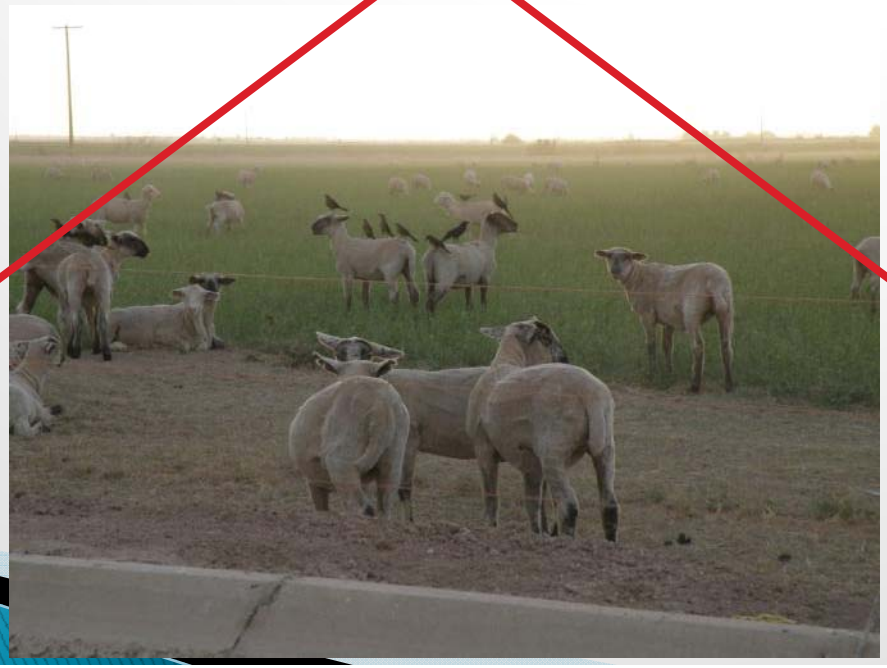


Food safety risks associated with sheep grazing in stubble fields

3. Measure the rate of inactivation of *E. coli* O157:H7 and *Salmonella* spp. as a function of such parameters as time, tillage practice, irrigation, ambient temperature, etc, and compare these estimates to the fate of commensal *E. coli*.

Materials and Methods

- “Band” (1,200 – 1,600 head) as experimental unit
- Pooled fecal samples
- 2 – 8 samples per band

Materials & Methods

- Recovery of *E. coli* O157:H7
  10g fecal slurry
  → enrichment broth (tryptic soy broth)
  → immunomagnetic separation (Dynal®)
  → 2 selective media (CT- SMAC, Rainbow agar)
  → immunoblotting
  → PCR (*rfb*E, *stx*1, *stx*2, *eae*, *hly, fli*C)

- Recovery of *Salmonella* sp
  10 g fecal slurry
  → enrichment broth x 2 (BPW and RV)
  → plate on XLD
  → biochemistry with TSI, citrate, urea, lysine

Results

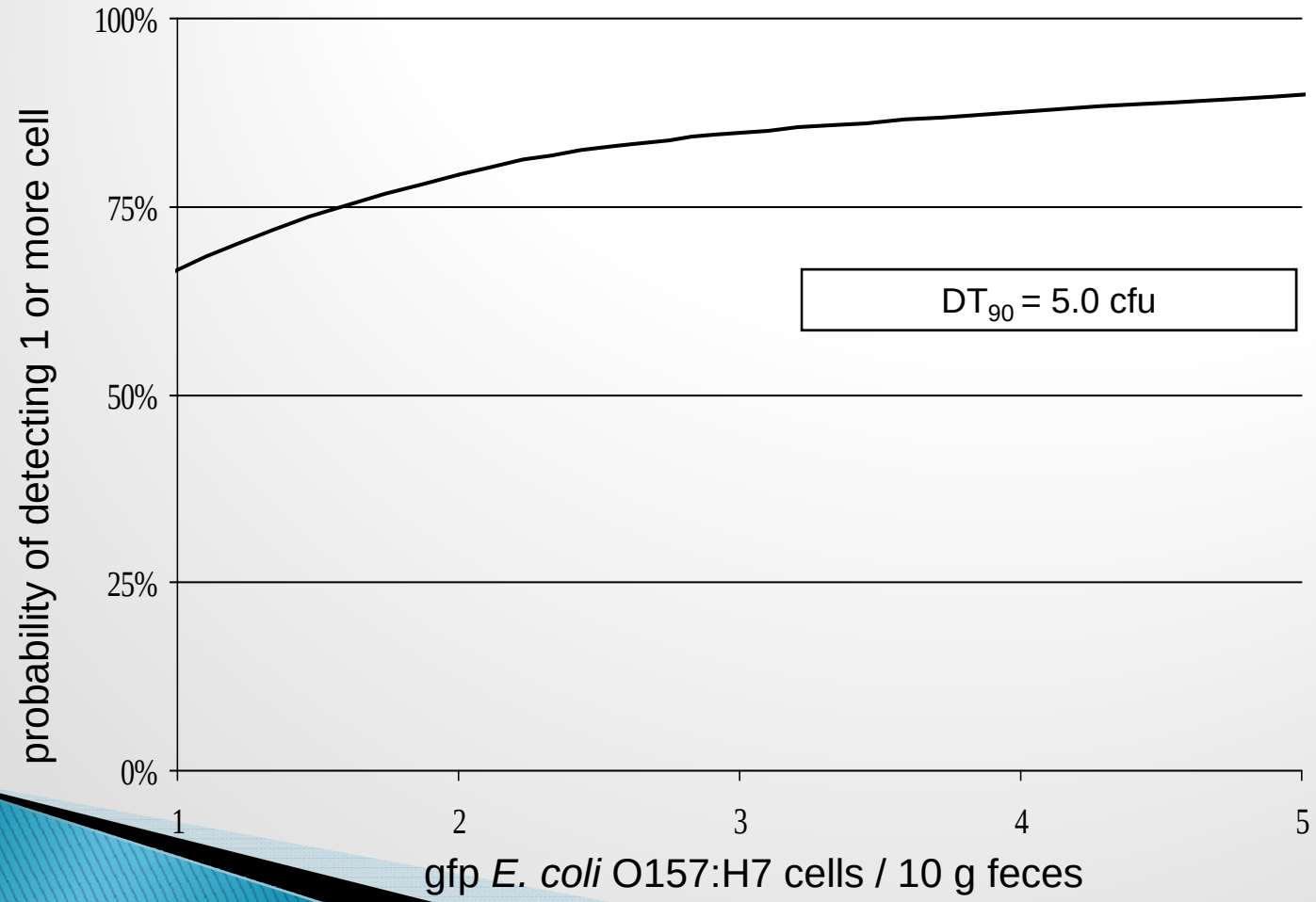
- Samples collected (weekly) from 11/12/09 to 03/09/10
- Total of 19 unique bands sampled
- 4 bands sampled repeatedly (7-9 times)
- Other bands sampled once

Results

- No samples positive for *E. coli* O157:H7
- 1 band positive for *Salmonella* sp. on two collection dates
- 6 bands positive for *Salmonella* sp. on one collection date
- Still waiting on serotype

Questions

- How sensitive is the test for *E. coli* O157:H7?



Results

- Commensal *E. coli*
  - Ranged from 5×10^5 to $>1 \times 10^{10}$ cfu/g
  - No association with presence of *Salmonella*
  - Survival study currently in progress

Conclusions (preliminary)

- Prevalence and intensity of shedding of *E. coli* O157:H7 and *Salmonella* sp. by sheep in this management system apparently low
- Further studies warranted to address the following questions:

Questions

- What about dust?

Questions

- What about birds?
- What about insects?

Acknowledgements

Veronica Villanueva
Jennifer Carabez
Sheep Producers

California Leafy Greens
Research Board

Questions?