Irrigation mediated transfer of *E. coli* O157:H7 from feces to lettuce, 2011 & 2012

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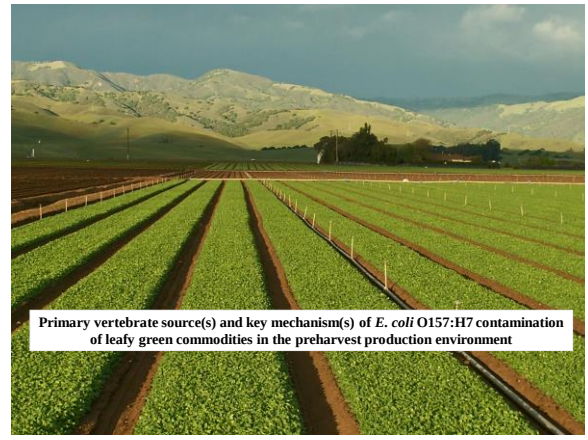
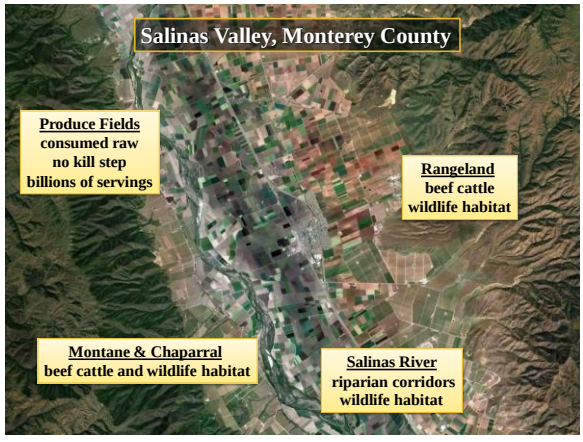


Primary vertebrate source(s) and key mechanism(s) of *E. coli* O157:H7 contamination of leafy green commodities in the preharvest production environment



Comparing livestock to wildlife shedding of key foodborne zoonotic pathogens

Cow-calf herds, central coastal CA, 2008-2010
***E. coli* O157 infection ranged from 0% to 10%**

Herd	pos	n	prev (%)
A	0	489	0.0
B	7	480	1.5
C	0	200	0.0
D	44	434	10.1
E	0	61	0.0
F	6	386	1.6
G	2	271	0.7
H	9	256	3.5
I	0	138	0.0
Total	68	2715	2.5

CA statewide survey of 20 cow-calf herds, 2012-2013

Butte, Contra Costa, Humboldt, Kern, Lassen, Madera, Modoc, Mono, San Joaquin, San Luis Obispo, Solano, Stanislaus, Tulare and Yuba County (14 counties)

1412 cows and calves

Prevalence (%) of fecal shedding (positive/total)

	Salmonella	*E. coli* O157	*Cryptosporidium*	*Giardia duodenalis*
Cow	0.4% (3/726)	5% (37/726)	9% (67/726)	23% (168/726)
Calf	0.15% (1/686)	5% (35/686)	20% (136/686)	42% (286/686)
TOTAL	0.3% (4/1412)	5.1% (72/1412)	14.4% (203/1412)	32% (454/1412)

Sierra Foothill Research & Extension Center, University of California

Buffer width (m)
0.1, 1.1, 2.1

Land slope (%)
5, 20, 35

RDM (kg/ha)
225, 560, 900, 4500

Wildlife and beef cattle from central coastal CA, 2008-10

***E. coli* O157:H7**

Feral pig	10/200	(5%)
Coyote	2/95	(2%)
Am. crow	5/93	(5%)
Cowbird	2/60	(3%)
Rabbit	0/108	(0%)
Skunk	0/63	(0%)
Tule elk	3/150	(2%)
Deer	0/447	(0%)
Rodents	2/1043	(0.2%)
Beef cattle	68/2715	(2.5%)

Salmonella enterica

wildlife	17/449	(3.8%)
cattle	1/795	(0.13%)

wildlife shedding was 30 times higher compared to cattle ($P<0.001$)

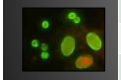
Prevalence = 5%

What is the probability of ≥1 positive bird in this group of 10 crows? 40%

Prevalence of pathogens in wild rodents in produce fields, central California, 2009-2011

n=8113 trap nights, 13% trap success (1071 rodents)

***E. coli* O157:H7 2/1,043 (0.2%)**
***Salmonella* 30/1,043 (2.9%)**
~50% *S. enterica* subsp. *arizonae* (IIIA)
~30% *S.* 1 6,8:d:-



Rodent species	*Cryptosporidium*	*Giardia*
CA parasitic mouse	11%	13%
Deer mouse	33%	27%
Dusky-footed wood rat	17%	17%
Total	**26%**	**24%**

Cryptosporidium: 4.3 × 10^8 oocysts/g feces; ~50% appear infectious for humans
Giardia appears not infectious to humans

Kilonzo et al., 2013, Appl. Environ. Microbiol. 79(20):6337–6344

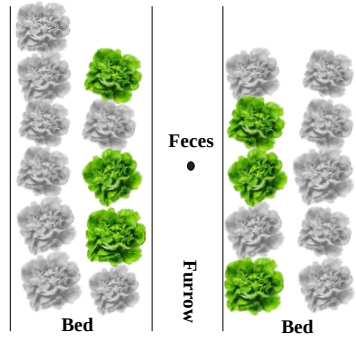
13

Winter precipitation runoff versus summer tail-water flows

Field trials of romaine lettuce: fecal transfer during irrigation ARS Salinas, California, July 2011

Atwill et al., 2015. J. Food Prot. 78(2):240–247

1.3×10^8 CFU of *E. coli* O157:H7 in 5 grams rabbit fecal slurry

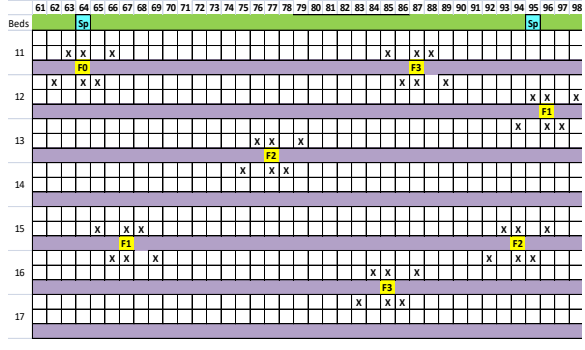


Timeline for Exp A & B irrigate on 7/14/2011

Exp A	days between fecal deposit and irrigate			irrigate					
	-3	-2	-1	0	1	2	3	4	
beds 11-30				F before irr, (S)					
			F	(S)					
		F		(S)					
	F			(S)					
	samples			168					
				spot fecal all 270 plants immeidately after irrigate					
				days between fecal spot and harvest					
Exp B				0	1	2	3	4	
				SF after irr, (S)					
beds 31-50				SF after irr	(S)				
				SF after irr		(S)			
				SF after irr			(S)		
				SF after irr				(S)	total
	samples			54	54	54	54	54	270
			total samples	222	54	54	54	54	438

F = fecal deposit, (S) = sample lettuce, SF = spot fecal

Time between fecal deposition and irrigation (-3, -2, -1, 0.1 dy), wind aspect, distances between lettuce to fecal and fecal to mean sprinkler.



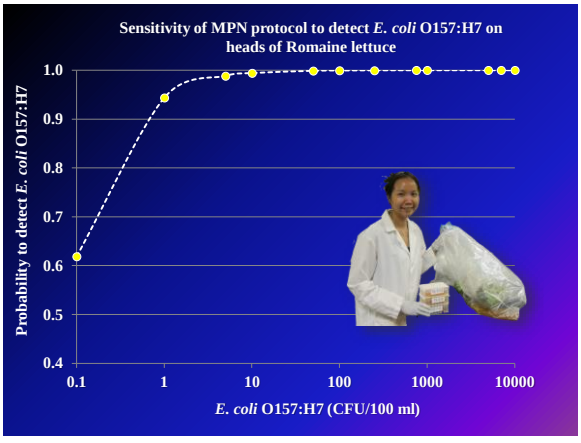
- Fecal deposit 5 gm / cluster
  - July 2011
    - rabbit only
  - July & October 2012
    - rabbit & pig
- 1 tech depositing fecal to minimize error

- Tootsie-Rolls (aka fecal patties)
  - ~1:1 fresh feces : PBS-*E. coli* O157
    - 2011 rabbit feces
    - 2012 rabbit & pig feces
  - Homogenized with stomacher
  - 5 gm field patties
  - 1 gm validation patties
    - 10-fold serial dilutions
    - 3 concentrations x 3 reps
    - No significant difference between field/lab

Lettuce sampling after irrigation

Bacterial MPN method

- High concentration assay
  - Detection limits
    - 340 – 3.5 × 10^{12} mpn/head
  - 2 × 6 mpn (1ml – 1 × 10^{-10})
    - 100-fold serial dilutions
- Low concentration assay
  - Detection limits
    - 2011: 1.8 – 550 mpn/head
    - 2012: 1.1 – 420 mpn/head
  - 4 × 3 mpn (5, 10, 50, 100ml)
    - 2011: 3 × 3 mpn (1, 10, 100ml)



2.5 hours of overhead irrigation using Nelson rotator emitters
1.25 to 3.85 mm applied

38% of lettuce heads within 70 cm (28 in) of scat had an average 7.4×10^3 *E. coli* O157:H7 after irrigation (1.3 to 230,000 MPN). Relative to the original load of 1.3×10^8 CFU of *E. coli* O157:H7 in 5 g, about 0.000057 (0.006%) transferred to the outer leaves of lettuce.

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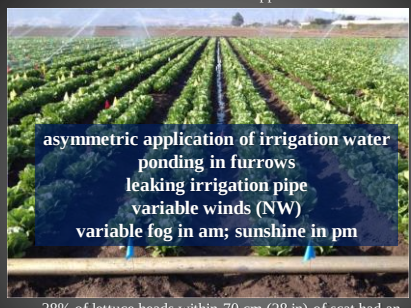

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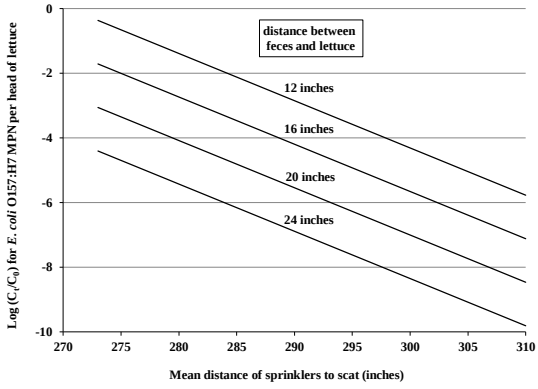
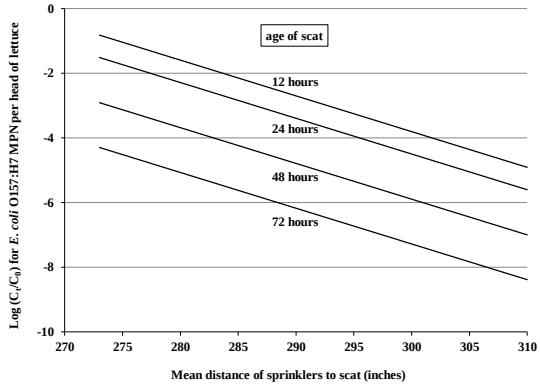
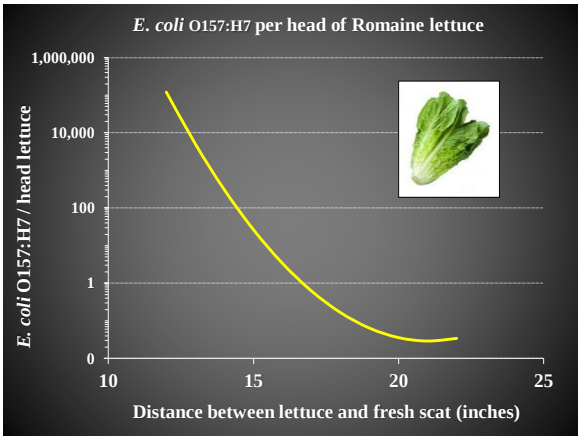
Negative binomial regression: $C_i = e^{(\alpha+\beta X_i)}$, $[\log(E(Y|X) = \alpha+\beta X]$
$\text{Log}(C_i/C_o)$, with C_o set at 1.3×10^8 MPN *E. coli* O157:H7/head

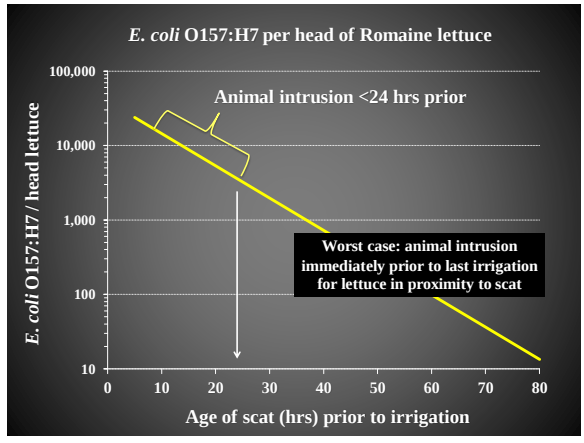
Factor	Coefficient	95% CI[b]	*P* value[b]
		Model 1 (AIC[c] = 264)	
Intercept	119.1	84.8, 153.3	<0.001
Distance between feces and lettuce (cm)	−0.305	−0.38, −0.23	<0.001
Distance between sprinklers and feces (cm)[d]	−0.133	−0.17, −0.09	<0.001
Aspect of wind relative to bed			
Downwind[e]	0.0		
Upwind	−6.88	−9.46, −4.31	<0.001
		Model 2 (AIC = 262)	
Intercept	87.9	42.5, 133.3	<0.001
Age of feces before irrigation (h)	−0.134	−0.18, −0.08	<0.001
Distance between sprinklers and feces (cm)[d]	−0.100	−0.16, −0.04	0.001
Aspect of wind relative to bed			
Downwind[e]	0.0		
Upwind	−4.87	−8.45, −1.30	0.008

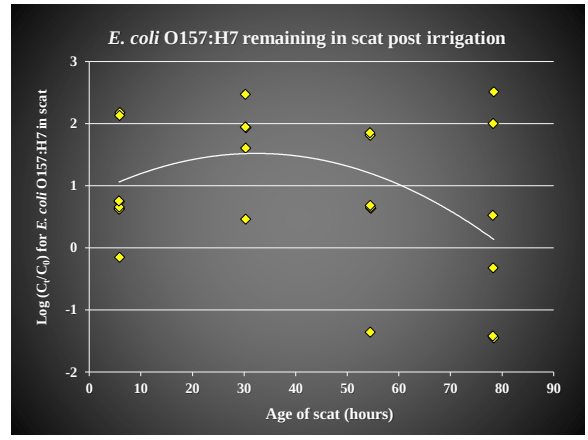
[a] The assay used for enumerating *E. coli* O157:H7 per head of lettuce was designed to estimate ≥340 MPN per head.
[b] The 95% confidence interval (CI) and *P* values adjusted for potential correlated bacterial data within each cluster of six adjacent heads of lettuce that surround a rabbit fecal deposit with an average bacterial load of 1.29×10^8 CFU of *E. coli* O157:H7.
[c] AIC, Akaike Information Criterion.
[d] Mean distance of the four sprinklers to the fecal deposit for each cluster of six heads of romaine.
[e] Referent category; lettuce head was downwind of the fecal deposit (lettuce located on north-facing, windward edge of bed).



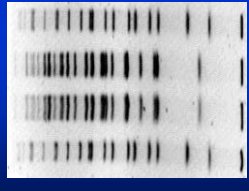








Rodent traps set each day of experiment
9 rodents trapped, 4 separate individuals
3 × for adult female tag 226 (lactating)
4 × for adult male tag 227 (positive on 3rd)



positive control

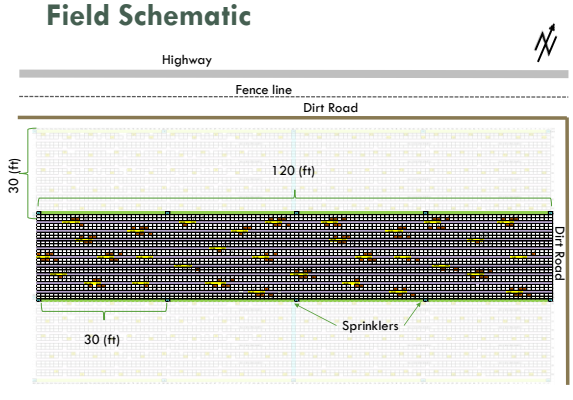
strain from male deer mouse #227

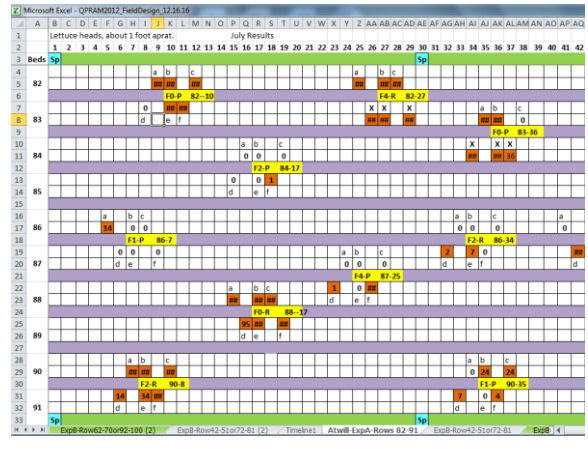
Field trials of romaine lettuce: fecal transfer during irrigation
ARS Salinas, California, July-August and October 2012

F=place fecal, (S)=sample lettuce

27-Jul	28-Jul	29-Jul	30-Jul	31-Jul	1-Aug
irrigate	days between fecal deposit and irrigate				irrigate
-5	-4	-3	-2	-1	0
					F b/f irr, irrig then (S)
				F	(S)
			F		(S)
					(S)
	F				(S)

Field Schematic

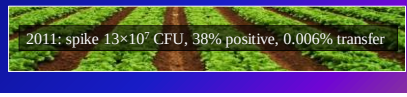




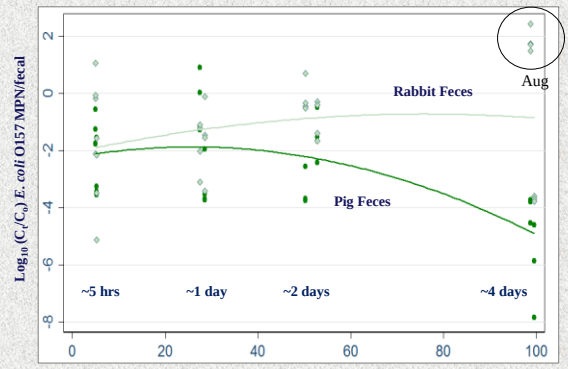
Irrigation-mediated fecal transfer onto Romaine lettuce
Salinas, CA 2012

	Percent pos. (p/n)	Percent transfer	Mean MPN/head	Range MPN/head	Fecal C_{start}	Fecal C_{end}	C_e / C_s
Aug 1, irrigate 1.25-3.85 mm							
Pig	49% (47/96)	0.001%	609	1-7,500	4.71×10^7	1.57×10^7	0.3
Rabbit	76% (73/96)	0.065%	23,640	1-550,000	55.8×10^7	126.1×10^7	2.3
Oct 21, irrigate 1.25-2.15 mm							
Pig	70% (67/96)	0.007%	12,393	1-550,000	17.8×10^7	0.64×10^7	0.04
Rabbit	79% (73/93)	0.005%	9,015	1-550,000	14.7×10^7	1.94×10^7	0.13

Median
0.00007%
60 MPN
C_{end} 12×10^7 MPN



***E. coli* O157:H7 remaining in feces post irrigation**



FSMA 2015: Minimize fecal contamination from domestic and wild animals in the produce field:

- Farmers are required to take all measures reasonably necessary to identify and not harvest produce that is likely to be contaminated.
- Requires all covered farms to visually examine the growing area and all covered produce to be harvested, regardless of the harvest method used.