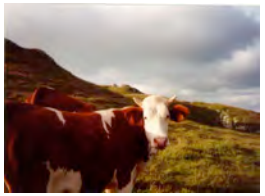
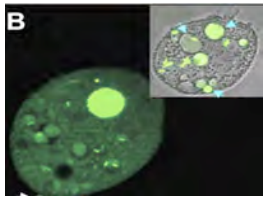
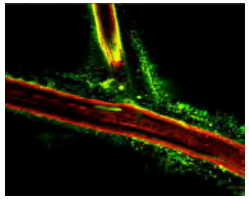




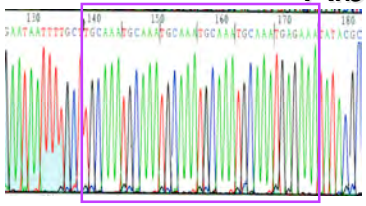


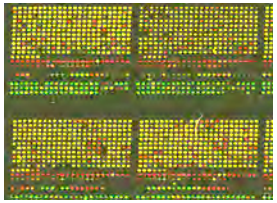
E.coli O157:H7 in Lettuce and Leafy Greens Intervention Assessment Model Workshop

What do we know about sources and environmental ecology?

Asilomar, California — November 13-15, 2006

Robert E. Mandrell, Ph.D.
Research Leader, Produce Safety and Microbiology Research Unit
USDA, Agricultural Research Service, Western Regional Research Center
800 Buchanan Street
Albany, CA 94710



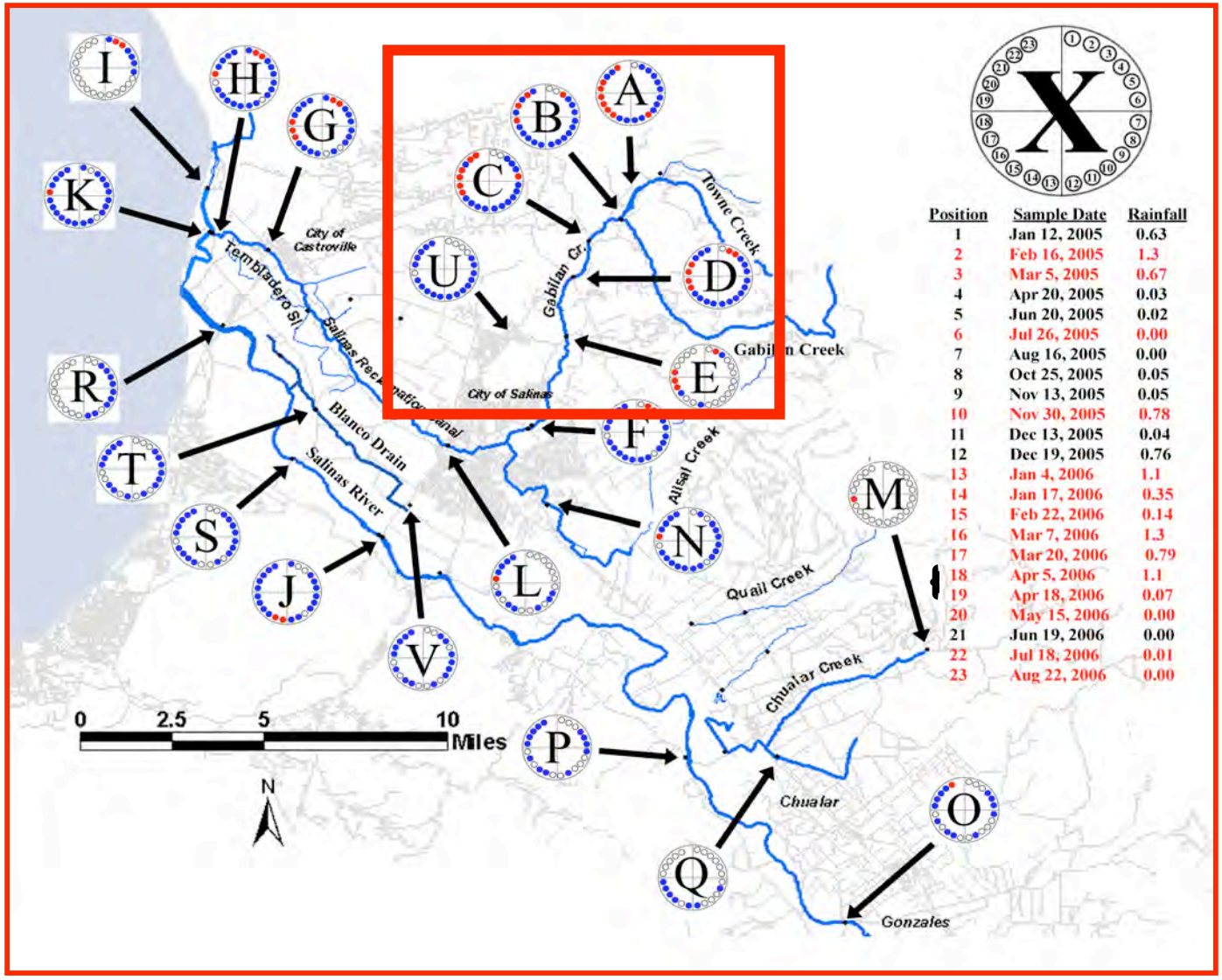


Sampling for *E.coli* O157:H7 in the Salinas Valley Watershed (initiated by J. Farrar, CDHS)

- Stimulated by identification of a farm that supplied leafy vegetables associated with 3 separate outbreaks
- Farm investigation: soil, water, plants, feces tested (>200 samples); only 1 strain of *E. coli* O157:H7 isolated, from sediment in a ditch
- Coordinated sampling with CCRWQCB, TMDL study (ARS and CDHS tested for Ec O157:H7)
- Two years of monthly, and sometimes more frequent, sampling

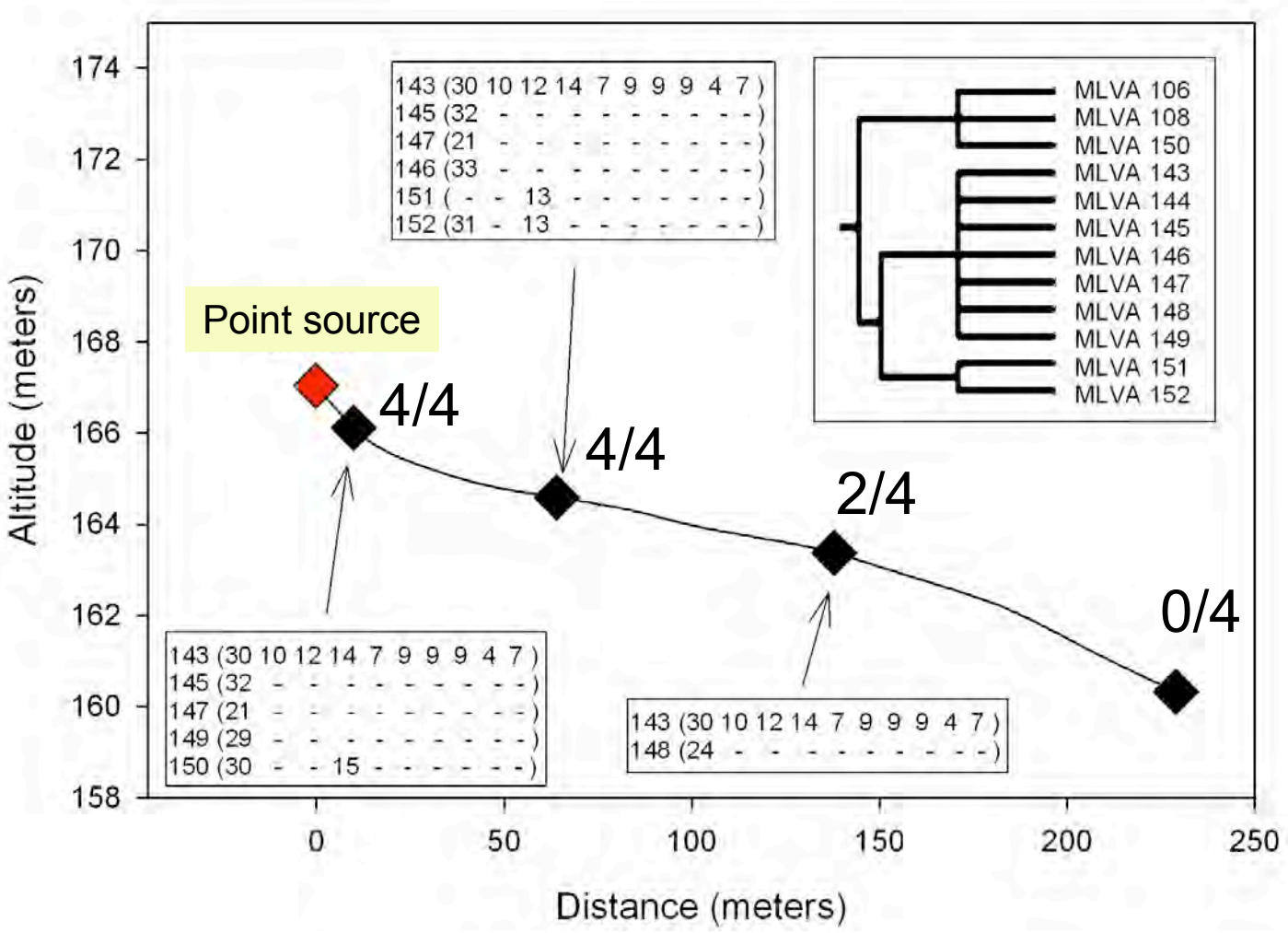
Sampling sites in the Salinas region

Summary of results of isolation of *E.coli* O157:H7 from the Salinas Valley Watershed



Position	Sample Date	Rainfall
1	Jan 12, 2005	0.63
2	Feb 16, 2005	1.3
3	Mar 5, 2005	0.67
4	Apr 20, 2005	0.03
5	Jun 20, 2005	0.02
6	Jul 26, 2005	0.00
7	Aug 16, 2005	0.00
8	Oct 25, 2005	0.05
9	Nov 13, 2005	0.05
10	Nov 30, 2005	0.78
11	Dec 13, 2005	0.04
12	Dec 19, 2005	0.76
13	Jan 4, 2006	1.1
14	Jan 17, 2006	0.35
15	Feb 22, 2006	0.14
16	Mar 7, 2006	1.3
17	Mar 20, 2006	0.79
18	Apr 5, 2006	1.1
19	Apr 18, 2006	0.07
20	May 15, 2006	0.00
21	Jun 19, 2006	0.00
22	Jul 18, 2006	0.01
23	Aug 22, 2006	0.00

Isolation of *E. coli* O157:H7 from multiple samples obtained at different sites on a creek near a potential point source (May 23-2006, low water flow)



Samples further downstream: no O157:H7 isolated

Comparison of watershed isolates PFGE profiles with submissions in the PulseNet database

MLVA types of Environmental strains	Watershed Source/ Location	PulseNet *XbaI*	% of PulseNet database	PulseNet *BlnI*	No. PulseNet Outbreaks/ Clusters
1	Water-G	EXHX01.1905	ND		
3	Water-H	EXHX01.0221	0.03		
13	Water-G	EXHX01.2122	ND		
15	Water -C	EXHX01.1271	0.36		
16	B (Cow feces)	EXHX01.0543	0.01		
17	U (Sediment)	EXHX01.0122	0.06	EXHA26.0354	
89,99	Water -A,D	EXHX01.0343	0.09	EXHA26.0569	
89,106,107,108,109, 122	Water-A,C,D,E	EXHX01.1709	ND	EXHA26.0569	
90	Water -J	EXHX01.2216	0.02	EXHA26.0684	
91	Water -J	EXHX01.2222	ND		
92	Water -J		ND		
93	**Water -J**	**EXHX01.0047**	**5.8**	**EXHA26.0015**	**9**
93,101,102,103	**Water - B,C,E,G**	**EXHX01.0200**	**0.8**	**EXHA26.0015**	**3**
94,95,96	Water -J	EXHX01.3099	0.03	EXHA26.0265	1
97,98	Water -A,G	EXHX01.2221	0.02		
100,105	Water - G,H,K,L,N	EXHX01.0200	0.8	EXHA26.0332	
104	Water-M	EXHX01.1031	0.12	EXHA26.0982	1

Closest MLVA types to spinach outbreak strains: None are identical by MLVA

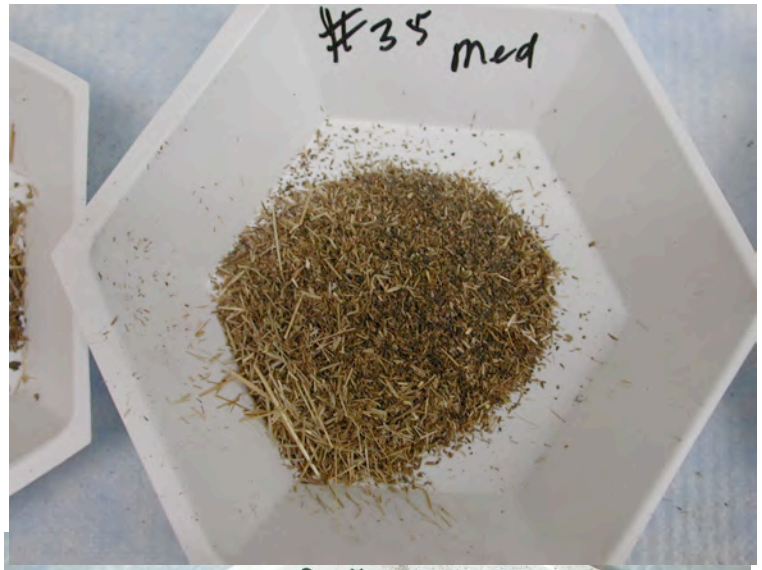
MLV A	Strain#	Source	Other info	Vhec1	Vhec2	Vhec3	Vhec4	Vhec5	Vhec6	Vhec7	O157-17	O157-19	O157-37
100	**RM5628**	**Water***	**3/18/06, 3/20/06, 4/18/06: TEMPRE, ALIAIR, OLSMON, RECVIC,**	**18**	**9**	**11**	**13**	**7**	**9**	**8**	**4**	**7**	**6.5**
105	**RM5675**	**Water***	**4/18/06 ALIAIR**	**18**	**9**	**11**	**14**	**7**	**9**	**8**	**4**	**7**	**6.5**
60	RM5200	Human	2005, Human, sporadic	**17**	9	11	15	7	9	8	4	7	6.5
115	RM5658	Human	2006, Human, sporadic	**16**	9	11	13	7	9	9	4	7	6.5
116	RM5652	Human	2006, Human, sporadic	15	9	11	18	7	9	9	4	7	6.5
163	RM 6011	Human	Spinach Outbreak 2006 WI	**16**	9	11	17	7	9	9	4	7	6
164	RM 6047	Human	Spinach Outbreak 2006 ID	**17**	9	11	17	7	9	9	4	7	6

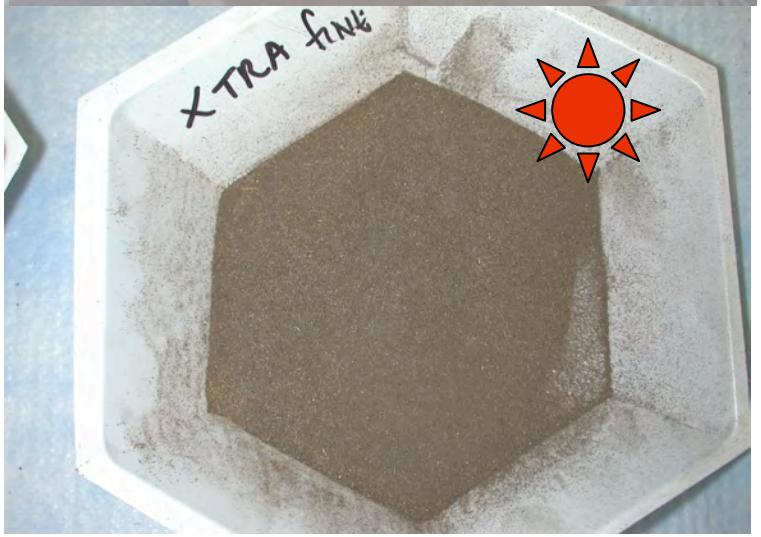
**Strains of these MLVA types match Matches PulseNet XbaI EXHX01.0200 and BlnI EXHA26.0332 profile*

Sample of “very dry dirt” from pasture:

EcO157 not isolated after 8 hr/42C enrichment;

isolated after enrichment at 21 hr/37C





USDA-CSREES-NRI grant award
Oct 1, 2006 to Sept 30, 2010, $1.17 million

"Ecology and epidemiology of *Escherichia coli* O157:H7 in fresh produce production regions of Salinas, California"

ARS (R. Mandrell, PD; M. Cooley)

UC Davis (R. Atwill, Co-PD; K. Tate; R. Larsen)

CDHS (L. Crawford, M. Jay-Russell, J. Mohle-Boetani)

Produce Industry (J. Gorny, United Fresh Produce Assoc.)

Letters of Support: A.G. Kawamura, Sec. of CDFA
K. Reilly, CDHS
H. Giclas, WGA
D. Gombas, UFPA (formerly IFPA)
J. Bogart, Growers-Shippers Assoc., CC
State Pub. Health Labs of CA, MN, OR, WA

(1) Determine risk factors for in-field contamination of lettuce with EcO157:H7

(2) Sampling water, soil, produce, livestock and wild animals

(3) Disseminate recommendations for risk reduction to prevent pre-harvest EcO157:H7

E. coli O157:H7 in the environment

How long does it survive?

Survival of *E. coli* O157:H7 in Soil, Seedlings, Feces

Islam et al, 2004 J Food Protection	• O157 persisted 154 to 217 d in soil amended with spiked compost; 77 d on lettuce, 177 d on parsley
Johannessen et al, 2005 Appl Environ Microbiol	• Uptake from manure to crisphead lettuce • Seedlings transplanted into manure-amended soil (10,000 O157 cells/g), then 50 d growth in greenhouse • Persisted in soil for at least 8 wks • No evidence of transfer to lettuce
Scott et al, 2006 J Food Protection	• Marked strain of O157:H7 in inoc. cattle feces • Survived 97 d in feces, 109 d in water • Survived 10 wk longer in cattle vs. lab inoc. culture

**Mukherjee et al, 2006
J Appl Microbiol**
	• **Child illness due to:**
	  • **O157:H7 in garden soil fertilized with cow manure**
	  • **This "naturally occurring" strain survived >69 days: survival at ambient temp. >> than at 4ºC**

Survival of *E. coli* O157:H7 in Water

McGee et al, 2002 J Appl Microbiol	• O157:H7 survived in "farm water" 14 d at <15ºC • Addition of bovine feces (1% w/v) increased survival to 24 d • "Farm water" may act as a vehicle for transport across a herd and into watershed
Maule, 2000 Symp Ser Soc Appl Microbiol	<u>Model systems</u> • O157 survived best in soil cores with rooted grass - 1 to 2 log decrease from 10^8 after 130 d • Inoculated cattle feces - Detectable at high levels for >50d • Lower survival in cattle slurry and in river water; detectable up to 10 and 27 d, respectively