Microbial water quality and produce safety



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California high value produce feeds the US

			CA production	Annual servings	% of US
Group	Commodity		(cwt)	(billions / yr)	total
Berries	Fresh strawberries	Ì	36,750,000	11.0	88.7
	Fresh raspberries		1,080,000	0.4	62.2
Grapes	Table grapes	N.	19,500,000	15.6	90.1
Leafy	Head lettuce	C	36,750,000	29.4	75.3
greens	Leaf lettuce	and the second s	10,557,000	8.5	85.9
	Romaine		17,614,000	14.1	71.5
	Spinach, fresh market ٰ		3,822,000	3.1	61.9
Nuts	Almonds, shelled	R	20,300,000	32.5	100
	Pistachios, in shell	r a	4,440,000	4.8	100
	Walnuts, shelled	A BO	2,720,000	4.4	100
Tomatoes	fresh market	6	12,425,000	5.0	40.9

Fall 2006 spinach outbreak of *E. coli* O157:H7 Product originated from a field in San Benito County, CA







Are these produce outbreaks the result of irrigation water, winter runoff, livestock grazing and/or wildlife? What is the biological source of pathogens and process of contamination? Many outbreaks in late summer or fall, so how does contamination occur?



Minimizing pathogen movement from livestock & wildlife to food and water



irrigation water, rangeland runoff

Developing beneficial management practices (BMPs): 1° goal is to match pathogen flux with local BMP efficacy



Key processes driving waterborne zoonotic transmission

- A. Vertebrate <u>pathogen loading</u>: *who sheds the pathogen?*
- B. Hydrological <u>transport</u>: *how are pathogens reaching water*?
- C. <u>Inactivation</u> kinetics: *can the pathogen survive long enough?*
- D. Inter-species infectivity: is the pathogen infectious for humans?

Comparing livestock to wildlife shedding of key waterborne zoonotic pathogens









Salinas Valley, Monterey County

Produce Fields raw food billions of servings

<u>Chaparral</u> wildlife habitat

<u>Rangeland</u> cow-calf wildlife habitat

<u>Salinas River</u> riparian corridors wildlife habitat Many outbreaks are in late summer or fall, so how does contamination occur?

Longitudinal survey, 4/2008 to 11/2011				
Soil samples	0.4%	(10/2450)		
Produce samples	0.0%	(0/2462)		
Water samples	0.4%	(1/242)		
Livestock	2.5%	(68/2715)		

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



<u>E. coli 0157:H7</u>				
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%)

<u>Salmonel</u>	2	
wildlife	17/449	(3.8%)
cattle	1/795	(0.13%)

wildlife risk 30 times higher compared to cattle (*P*<0.001)

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

<u>Herd</u>	pos	<u>n</u>	<u>prev (%)</u>
A	0	489	0.0
B	7	480	1.5
С	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

Phylogenetic tree of *E. coli* O157:H7 spinach outbreak and cattle isolates from outbreak region, central California



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	<i>E. coli</i> 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)



From Rincon Creek up to Aptos Creek 23 rivers, creeks or their estuaries

CCRWQCB

April 2009 to April 2010

E. coli O157 6/251 = 2.4%

Salmonella 78/251 = 35% 1.3 MPN/100 ml



Recall <<1% cow-calf shed *Salmonella*; 2-4% in wildlife

Cryptosporidium from CA beef cattle in this study appear to have <u>low to no infectivity</u> for humans

	C. andersoni	C. bovis	C. ryanae	C. parvum
Cow	0	1	18	0
Calf	1	18	43	0
Total	1 (1.2%)	19 (23.5%)	61 (75.3%)	0 (0%)

Giardia duodenalis from CA beef cattle in this study <u>appear to have low to no infectivity</u> for humans

	Assemblage E	Assemblage C	Unknown
Cow	56	8	2
Calf	128	7	4
Total	184 (90%)	15 (7%)	6 (3%)

Prevalence of pathogens in wild rodents from produce fields and cattle ranches, central California



E. coli O157:H7 2/1043 (0.2%) *Salmonella* 30/1043 (3.0%)



Rodent species	Cryptosporidium	Giardia
CA parasitic mouse	11%	13%
Deer mouse	33%	27%
Dusky-footed wood rat	17%	17%
TOTAL	30%	26%

Crypto appears human infectious, Giardia appears not



<u>Concentration of *Cryptosporidium* in infected deer mice</u> over 50 million oocysts / gram of feces

or

2,500,000 oocysts per fecal pellet (5 mg)!!





Winter precipitation runoff versus summer tail-water flows

<u>cow-calf ranches</u> 1.4 to 7 deer mice/acre

0.05 to 2.7 cattle/acre

produce field 1 to 34 deer mice / acre (mean of 8.5 mice / acre) 0 cattle in produce field Environmental loading of *Cryptosporidium rubeyi* and other *Cryptosporidium* species by California ground squirrels on rangeland, Kern County, CA



When wildlife congregate then food safety risks are magnified



Prevalence = 5%

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

Prob
$$(X \ge 1 \text{ positive})$$
 for 10 crows
Using the binomial distribution,
 $\binom{n}{x}(p)^{X}(1-p)^{n-X}$

Prob = 40% for \geq 1 crow with *E. coli* O157, n=10

What if 20 crows visit the field? 64% chance for \geq 1 bird with *E. coli* O157

n=30, prob=79% n=40, prob=87%

So how did the *E. coli* cross the road and contaminate the produce?



Randomized field trials of romaine lettuce Salinas Valley, 2011 & 2012





Add in 2 hours of irrigation



20 to 30% of nearby heads of lettuce contaminated with *E. coli* O157:H7

E. coli O157:H7 per head of Romaine lettuce



E. coli O157:H7 per head of Romaine lettuce



Attenuated *E. coli* O157 survival in pig feces July 2012 field trial



Exclude wildlife by fencing, trapping and habitat modification



Best theory:

- wildlife intrusion, likely nocturnal
- combine with foliar irrigation
- combine with difficult biosecurity during harvesting
- lack of a kill step during processing
- product consumed raw by millions of consumers

multiple independent variables sporadically combine to create full connectivity

Dr. Ken Tate: developing beneficial management practices: 1° goal is to match pathogen flux with BMP efficacy



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