

## Historical

- First characterized in late 1800s (Theodor Escherich)
- Recognized as common to intestinal tract of humans and animals

## *E. coli* overview

- Most common (up to  $10^8$ /g) facultative bacterium in intestines of mammals & birds — almost all nonpathogenic.
- Until *E. coli* O157:H7 (& other EHEC?), *E. coli* pathogenic for humans were human-specific.

## Characteristics

- Member of *Enterobacteriaceae*
- Coliform: ability to ferment lactose with acid and gas
- Facultative, gram-negative rod
- Acid and gas from glucose

## Characteristics

- Temperature range: 7–8 to 44–46°C, optimum 37°C
- pH range 4.4–9.0, optimum 6–7
- Water activity: minimum 0.95; optimum 99.5

## Taxonomy

- Somatic antigen O (outer-membrane protein): >200 O antigens (173 defined?)
- Capsular antigen: 103 K antigens
- Flagellar antigen H: 56 H antigens (note “NM”)
- Fimbriae/pili
- Over 700 serotypes recognized

Food-associated illness: six virulence groups currently recognized

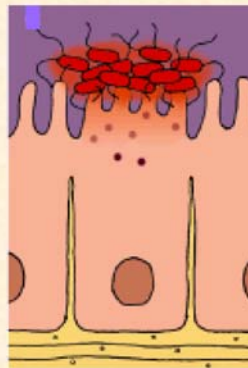
- Enteroaggregative (EA<sub>gg</sub>EC)
- Enteroinvasive (EIEC)
- Enteropathogenic (EPEC)
- Enterotoxigenic (ETEC)
- Enterohemorrhagic (EHEC)
- Diffusely adherent (DAEC)

## Enteroaggregative (EAEC)

- Unclear whether cause foodborne illness
- Persistent diarrhea in children
- Diarrhea watery and mucoid.
- Up to 30% grossly bloody stools.

## EAEC - 2

- EAEC bind in clumps (aggregates) to cells of the small intestine and produce toxins.
- Virulence factors include aggregative adherence and heat-stable enterotoxin; plasmid-mediated



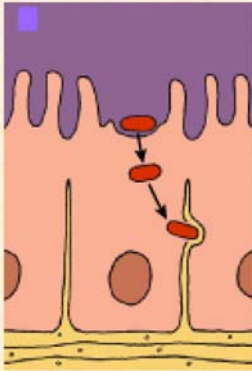
Enteroaggregative *E. coli* (EAEC) form a biofilm and secrete cytotoxins

## Enteroinvasive (EIEC)

- Acute dysenteric diarrhea
- EIEC invade cells in the colon and spread laterally, cell to cell.
- Virulence factors include cell invasion and intracellular multiplication; plasmid and chromosomally mediated.

## EIEC - 2

- Est. infectious dose:  $10^8$
- Incubation: 8–24 h, mean 11 h
- Profuse diarrhea or dysentery, chills, fever, headache, muscular pain, abdominal cramps
- Duration: days to weeks



**Entero-invasive *E. coli* (EIEC) invade enterocytes and move laterally in epithelium**

### Enteropathogenic (EPEC)

- Children under age of 1 year
- Traveler's diarrhea
- Acute and/or persistent profuse watery diarrhea, vomiting, fever
- Uncommon in temperate climates with good hygienic standards

### EPEC - 2

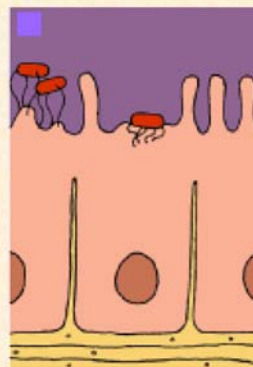
- Immunity thought to play a role in low incidence. WHO report suggests that 50% of children carry antibodies which may lead to immunity.

### EPEC - 3

- EPEC attach to intestinal mucosal cells causing cell structure alterations (attaching and effacing). EPEC cells invade the mucosal cells.
- Virulence factors include attaching and effacing lesions, localized adherence mediated by bundle-forming pili; plasmid- and chromosomally mediated

### EPEC - 4

- Est. infectious dose:  $10^5$ – $10^{10}$
- Incubation: 17–72 h, mean 36 h
- Symptoms described in Table 4
- Duration: 6 h–3 days, mean 24 h



**Entero-pathogenic *E. coli* (EPEC) induce pedestal formation & attach like EHEC**

## Enterotoxigenic (ETEC)

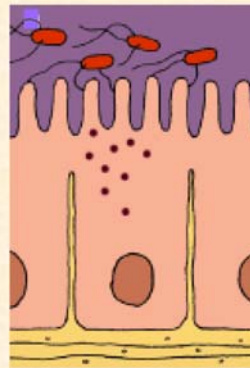
- Diarrhea, vomiting, and fever
- Traveler's diarrhea
- Profuse watery diarrhea without blood or mucus

## ETEC - 2

- ETEC adhere to the small intestinal mucosa and produce toxins that act on the mucosal cells.
- Virulence factors include adherence (**host-species specific**) and heat-stable or heat-labile toxins; plasmid- and chromosomally mediated.

## ETEC - 3

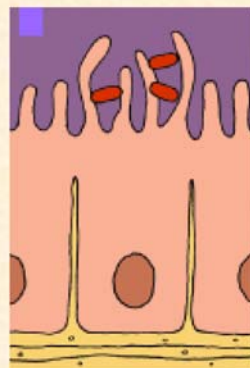
- Est. infectious dose:  $10^8$ – $10^{10}$
- Incubation: 8–44 h, mean 26 h
- Symptoms described in Table 4; severe version like cholera
- Duration: 3–19 days



Enterotoxigenic *E. coli* (ETEC) “inject” toxins into enterocytes

## Diffusely adherent (DAEC)

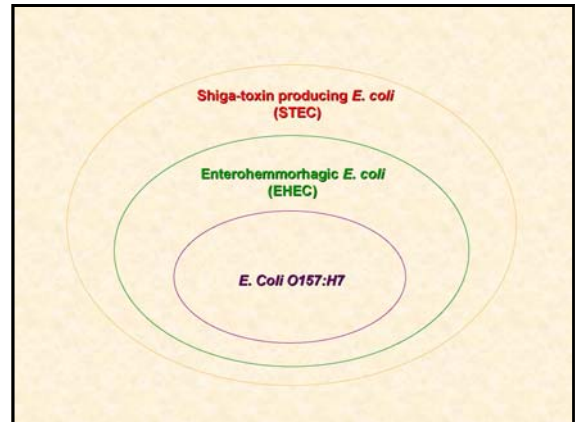
- Childhood diarrhea
- Fimbrial and non-fimbrial adhesins identified; plasmid- and chromosomally mediated.
- Cause elongation of microvilli



Diffusely adherent *Escherichia coli* cause elongation of microvilli

## Enterohemorrhagic (EHEC)

- Shiga-toxin production reported in over 100 *Escherichia coli* serotypes (STEC).
- Enterohemorrhagic *E. coli* (EHEC) strains are a subgroup of STEC that are associated with serious disease in humans.



## Background

- *Escherichia coli* O157:H7 is the most frequently reported EHEC and an important cause of infectious bloody (enterohemorrhagic) diarrhea and hemolytic uremic syndrome (HUS) in North America.
- An estimated 73,000 human cases and 61 deaths occur in the United States each year (Mead et al, 1999).

## Escherichia coli O157:H7 An Evolving Story

Year	Event
1982	<i>E. coli</i> O157:H7 first recognized as a human pathogen
1993	Large western states outbreak due to undercooked hamburgers served as a fast-food restaurant chain
1994	<i>E. coli</i> O157 added to the list of nationally reportable diseases
1995	First documented lettuce-associated outbreak
1996	Outbreak associated with unpasteurized apple juice affecting the U.S. and Canada
1997	Multi-state outbreak linked to eating raw sprouts grown from contaminated seeds

## Escherichia coli O157:H7 An Evolving Story

Year	Event
1998	FDA issues guidance document for produce safety
2000	Outbreaks of <i>E. coli</i> O157:H7 infections among children after direct contact (petting) with farm animals
2001	<i>E. coli</i> O157:H7 genome sequence completed and published in Nature
2003	Two separate outbreaks linked to consumption of fresh-cut produce (lettuce and spinach) in California
2006	Spinach and shredded lettuce outbreaks traced to CA fields; first identification of outbreak strains on farms
2007	Marketing Agreement/Metrics program initiated in California

## Background

- Virulence factors include
  - Attaching and effacing adherence
  - Shiga toxins
  - Hemolysin
- Plasmid- and chromosomally mediated.

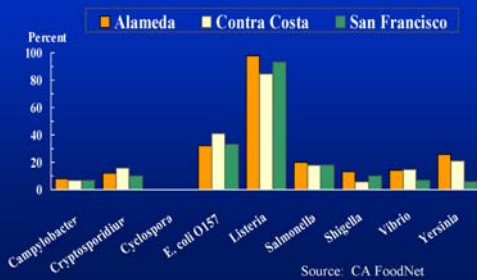
## Background

- Shiga toxins are a family of related phage encoded cytotoxins divided into two major groups:
  - *stx1* has 99% homology to shiga toxin of *Shigella dysenteriae* (immunologically identical)
  - *stx2* has 55% homology to *stx1* (immunologically distinct; multiple variants)
- STEC strains may have *stx1*, *stx2* or both genes.

## Background

- Hemolytic uremic syndrome (HUS) in some cases (<5 yr?)
- More rarely, thrombotic thrombocytopenic purpura (TTP)

## Percent of Cases Hospitalized in California FoodNet by Pathogen, 1996-2003



## Background

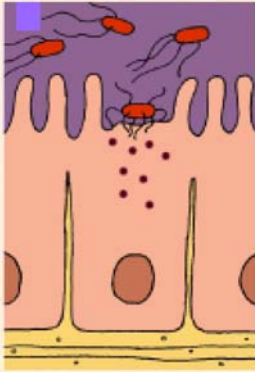
- EHEC attach to and efface mucosal cells and produce toxin(s) which cause an irreversible inhibition of protein synthesis in eukaryotic cells.

## Background

- A subunit shows enzymatic activity
- B subunit binds to receptors on the surface of some tissues (kidney).
- **Predominant** serotype: O157:H7

## Background

- Est. infectious dose: <100 (10?)
- Incubation: 3–9 days, mean 4 days
- Symptoms described in Table 4; hemorrhagic colitis, HUS, TTP
- Duration: 2–9 days, mean 4 days

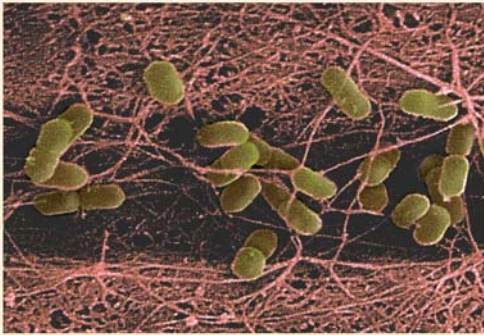


**Enterohemorrhagic *E. coli* (EHEC) attach intimately to enterocytes and inject toxins**

## **EHEC reservoir, vehicles**

- Livestock (cattle, sheep, goats)
- Wildlife (deer, rabbits, wild pigs)
- Foods often undercooked beef
- Contaminated RTE foods — meat to other food
- Naturally contaminated produce (unpasteurized juices, sprouts, leafy greens (lettuce, spinach))

## ***E. coli* O157:H7 in meat**



## ***E. coli* O157:H7 Testing**



## ***E. coli* O157:H7 Detection**

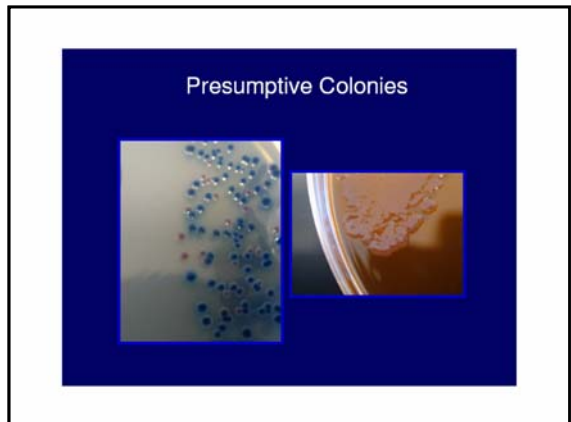
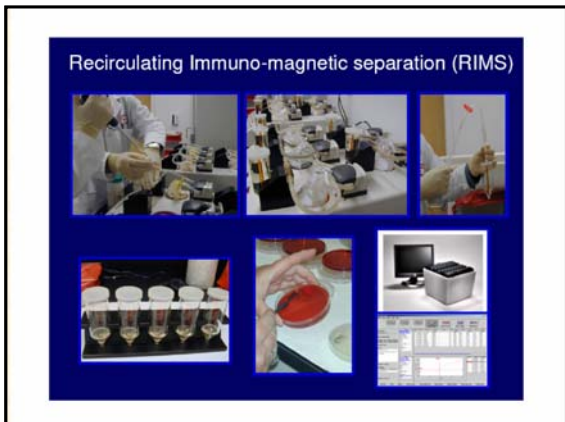
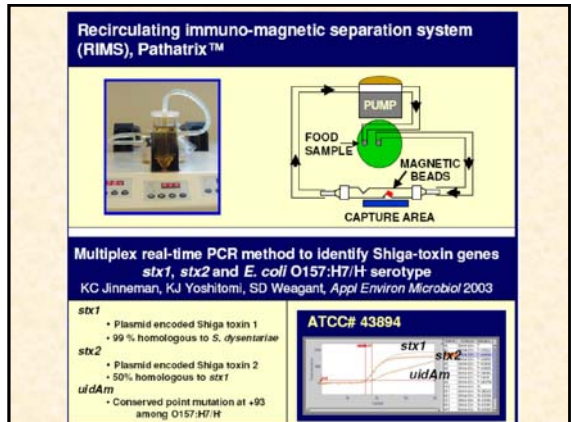
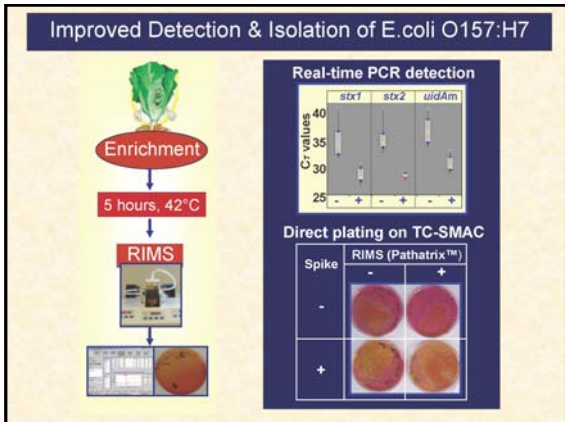
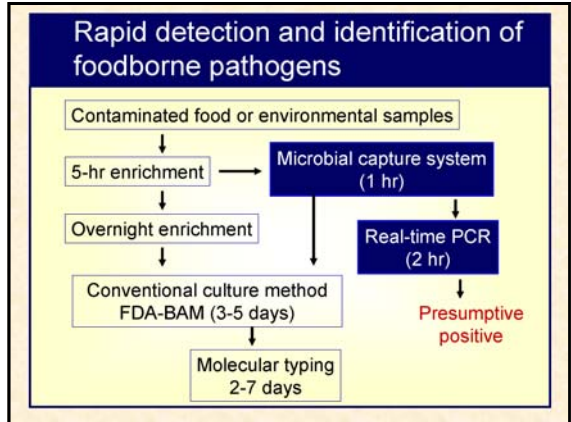
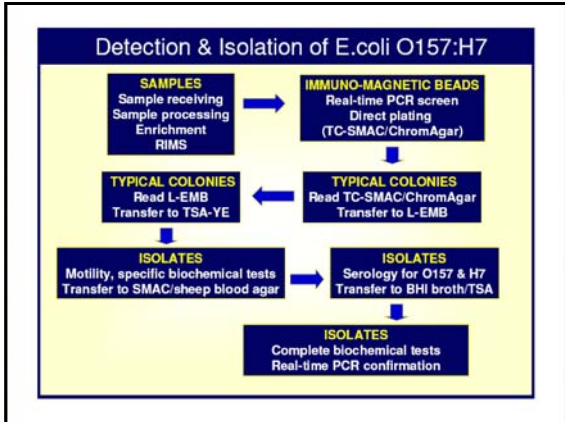
Sample Processing



## ***E. coli* O157**

**DIP STICK EXAMS (Fiction!)**



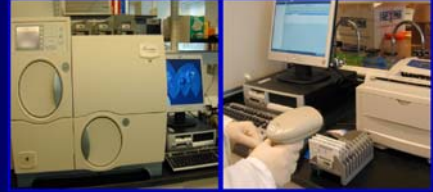




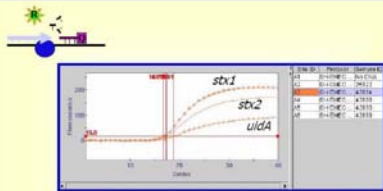
## Serotyping



## Biochemical Analysis



## Real-time PCR Confirmation



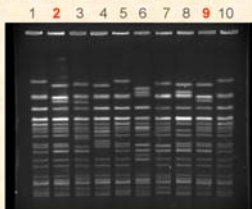
Identification of Shiga-toxin genes and *E. coli* O157:H7/H-

## DNA Fingerprinting of Foodborne Pathogens

- “Outbreak strains” are tracked through “PulseNet,” the Centers for Disease Control and Prevention’s (CDC) national DNA fingerprinting database for foodborne pathogens

## Pulsed-field gel electrophoresis (PFGE)

- Photo of 10 *E. coli* O157:H7 PFGE patterns posted on PulseNet (matching outbreak strains shown in lane 2 and 9)



## Multi-locus Variable Number Tandem Repeat Analysis (MLVA)

- Compare DNA sequences (4 letter code ATCG) of selected genes in the bacteria
- Below: *E. coli* O157:H7 (locus 2). Note that the number of “tandem repeats” of “ACC” match for strain B, C, and D (6 repeats), but not strain A (4 repeats)

Strain A      ggtaaccggtaaagcgcACCACCACCACCTtgacactgccggttg  
 Strain B      ggtaaccggtaaagcgcACCACCACCACCACCACCTtgacac  
 Strain C      ggtaaccggtaaagcgcACCACCACCACCACCACCTtgacac  
 Strain D      ggtaaccggtaaagcgcACCACCACCACCACCACCTtgacac

## Outbreak #1

- Hamburgers 1993, US Northwest
- Estimated 1–15 cells/g or 40–600 cells per raw patty
- >700 people affected, 195 hospitalized, 55 HUS or TTP, 4 deaths; *approximately 10% cases person-to-person*

## Outbreak #1 (cont.)

- Contributing causes: undercooking
- Control: adequate cooking, avoiding cross-contamination, sanitation

## Regulatory Changes

- 1988 FSIS policy change for precooked patties to 160°F (71°C) from 140°F (60°C)
  - Modified in 1990 to include lower temp/longer time options

## Regulatory Changes - 2

- 1993 Food Code changed to 155°F (68°C) for 15 sec
  - Consumer recommendations 160°F (71°C)

## Regulatory Changes - 3

- 1996 Meat HACCP regulations
  - *E. coli* O157:H7 defined as an “illegal adulterant” in raw ground beef
- 1999 — Irradiation approved for red meats

## “Illegal adulterant”

- One sample of ground beef tested—if O157 detected, entire lot condemned.
- No retesting permitted.

## Recent outbreaks

- Spinach, September 2006
  - 205 cases in 26 states and Canada
  - Traced to processor/farms in the California Central Coast
- Shredded head lettuce (tacos), Nov-Dec 2006
  - 81 cases in 3 states
  - Traced to Central Valley
- Shredded head lettuce (tacos), Nov-Dec 2006
  - Traced to Central Valley

## Spinach-Associated Outbreak Timeline

- Sept 8: Wisconsin PHD notifies CDC of a small cluster of HUS cases with matching PFGE. Oregon SHD identifies a cluster of 2 cases of *E. coli* O157 with matching PFGE.
- Sept 13: Clusters in Wisconsin now at 17 cases and 6 cases in Oregon. CDC and Wisconsin both notify FDA.
- Sept 14: Nationwide conference call. Thirteen states report 45 cases, 8 HUS, 1 death. FDA issues consumer advisory for fresh, bagged spinach.

## Spinach-Associated Outbreak Timeline

- Sept 16: FDA advises consumers not to eat "fresh spinach"
- Sept 18: CalFERT deployed to 9 farms.
- Sept 21: Positive spinach leftover sample in NM matches outbreak strain
- Oct 12: First samples (cattle feces collected 9/27) positive for outbreak strain at a single ranch. Joint FDA-CDHS press release
- Oct 26: Press conference announcing outbreak strain found in wild pig colonic feces and river water at the same ranch

## 2006 Spinach Associated Outbreak

- ✓ *Escherichia coli* O157:H7
- ✓ 205 illnesses
- ✓ 26 states report illnesses
- ✓ Canada reports illnesses
- ✓ 104 (51%) hospitalizations
- ✓ 31 (15%) patients with HUS
- ✓ 3 (1%) deaths

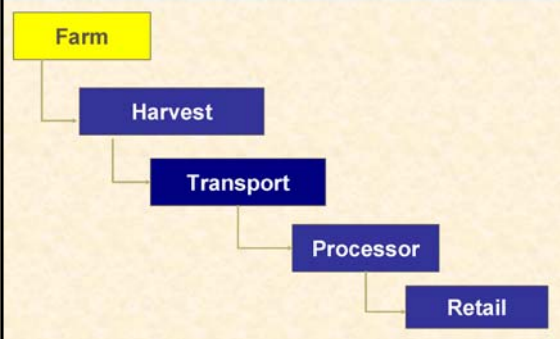
Traced from patients to bagged fresh spinach grown in the California Central Coast



## Environmental Sampling during the *E. coli* O157:H7 Spinach Outbreak 2006



## Principles of Environmental Investigations Into Food Product Microbial Contamination



## Potential Risk Factors for Pre-Harvest Microbial Contamination of Fresh Leafy Greens



### Contaminated Water, Flooding

*"From both sides of the valley little streams slipped out of the hill canyons and fell into the bed of the Salinas River. In the winter of wet years the streams ran full-freshet, and they swelled the river until sometimes it raged and boiled, bank full, and then it was a destroyer. The river tore edges of the farm lands and washed whole acres down..."*

*John Steinbeck, East of Eden*



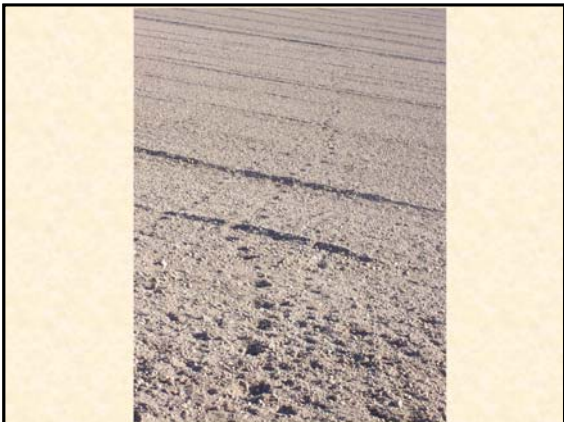
What are the best samples to collect during a farm investigation?

How many?



### Wells and Holding ponds



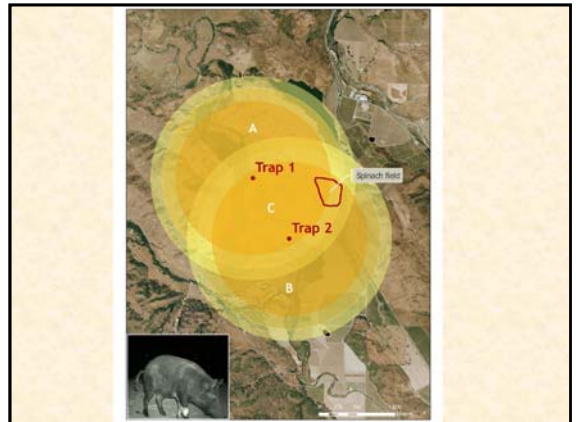




### Remote-sensing infrared digital surveillance camera



68 individual wild pigs observed within one mile from the spinach field based on physical sightings, live-capture, and cameras



## Harvesting baby spinach



### Samples from the Spinach Outbreak 2006

Animal feces: bird, cattle, coyote, deer, dog, horse, sheep/goat, wild pig (feces, colonic feces, buccal swabs, rectal-anal swabs, tonsils)

•Compost: chicken pellets

•Environmental swabs

•Field products: spinach, leafy greens

•Moore swabs

•Soil/sediment

•Water: well, river, creeks, ponds, cattle troughs

### Samples from the Spinach Outbreak 2006

- 879 total samples collected from 3 processing facilities, 2 harvesters, and 9 farms/ranches and surrounding watersheds
- Sampling narrowed to a single processing facility (San Benito County) and 4 farms, Ranches A, B, C, and D, in San Benito and Monterey Counties

### Samples from the Spinach Outbreak 2006

- No *E. coli* O157:H7 isolated from the processing facility or harvesting equipment
- *E. coli* O157:H7 isolated from 4 farms/ranches and the San Benito river (20 miles upstream of Ranch A)
- 28 isolates matched the “outbreak strain” by PFGE at a single ranch: cattle feces, wild pig feces/tissue, river water and sediment, pasture dirt

### Positive Samples from Spinach Outbreak 2006

#### Ranch A

- Cattle feces (26/77, 34%); 15 matches
- Wild pig colonic feces or feces from ground (13/87, 15%); 8 matches
- San Benito river, water (3/49, 6%), 2 matches
- San Benito river, sediment (2/14, 14%), 2 matches
- Soil from cattle pasture (1/6, 17%), 1 matches
- Moore swab, San Benito river 20 miles upstream (2/2, 100%)



## Positive Samples from Spinach Outbreak 2006

### Ranch B

- Cattle feces (9/21, 43%)
- Water, cattle trough (1/4, 25%)

### Ranch C

- Soil in cattle pasture (4/25, 16%)

### Ranch D

- Moore swab, Pajaro river (1/2, 50%)

## Spinach Outbreak 2006

### Conclusions

- Potential pre-harvest environmental risk factors
  - Large wild pig population in close proximity to cattle and spinach fields
  - Proximity of irrigation wells to surface waterways exposed to feces from cattle and wildlife

## Spinach Outbreak 2006

[Link to the final report from FDA and CDHS](http://www.dhs.ca.gov)

<http://www.dhs.ca.gov>

## Spinach Outbreak 2006

### Acknowledgments

Members of the California Food Emergency Response Team (Cal-FERT, a joint FDA-CDHS emergency response team that completes environmental foodborne outbreak investigations)

Richard Gelting, CDC, Atlanta GA

Jerry Wiscomb, USDA Wildlife Services, Sacramento, CA

Robert Mandrell, Mike Cooley, Diana Chao, USDA Agricultural Research Service, Western Regional Research Center, Albany, CA

Central California Coast growers, ranchers, and property owners