Tauxe #12

Emerging Foodborne Infections: The Produce Story

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Outbreaks of produce - associated foodborne infections

- 1998 *Yersinia pseudotuberculosis*, 47 cases, iceberg lettuce
- 1999 *Salmonella* Newport, 78 cases, mangoes
- 2003 *Salmonella* Muenchen, 58 cases, pre-cut melon
- 2004 *Salmonella* Bovismorbificans, 12 cases, alfalfa sprouts
- 2004 *Salmonella* Javiana, 492 cases, Roma tomatoes

- Currently:
  - *E. coli* O157:H7, 11 cases, precut Romaine lettuce
  - *E. coli* O157:H7, 32 cases, Fruit salad (melons?)
  - *E. coli* O157:H7, 12 cases, fresh apple cider

- Increasingly recognizing a problem in fresh produce

CDC

Produce-associated outbreaks reported to foodborne outbreak surveillance system

- Most foodborne outbreaks detected, investigated and controlled by local and state health departments
- Since 1973, CDC collects reports of outbreaks investigated
  - Reporting is voluntary and incomplete
  - Definition of an outbreak:

    2 or more cases of a similar illness resulting from the ingestion of a common food
  - Data collected: No of cases, implicated food, etiology
  - Before 1998: received reports of 400-600/year
  - Revised surveillance: 1200/year since then

Foodborne outbreaks related to fresh produce, 1973-1997

- Fresh produce defined as: uncooked produce items, or "salad" without eggs, cheeses, seafood or meat

- 1973-1997
  - 190 foodborne outbreak linked to fresh produce
  - 16,058 illnesses
  - 598 hospitalizations
  - 8 deaths

- 3.2 % of all outbreaks of determined source
- 6.2 % of those outbreak-associated cases

Sivapalasingam. J Food Protection 2005;67:2342-53

CDC

Foodborne outbreaks related to fresh produce, 1973-1997: Trends in burden

	1970's	1990's
Number of outbreaks/yr	2	16
Median cases/outbreak	21	43
% of OB of known vehicle	0.7%	6%
% of outbreak associated cases	0.6%	12%

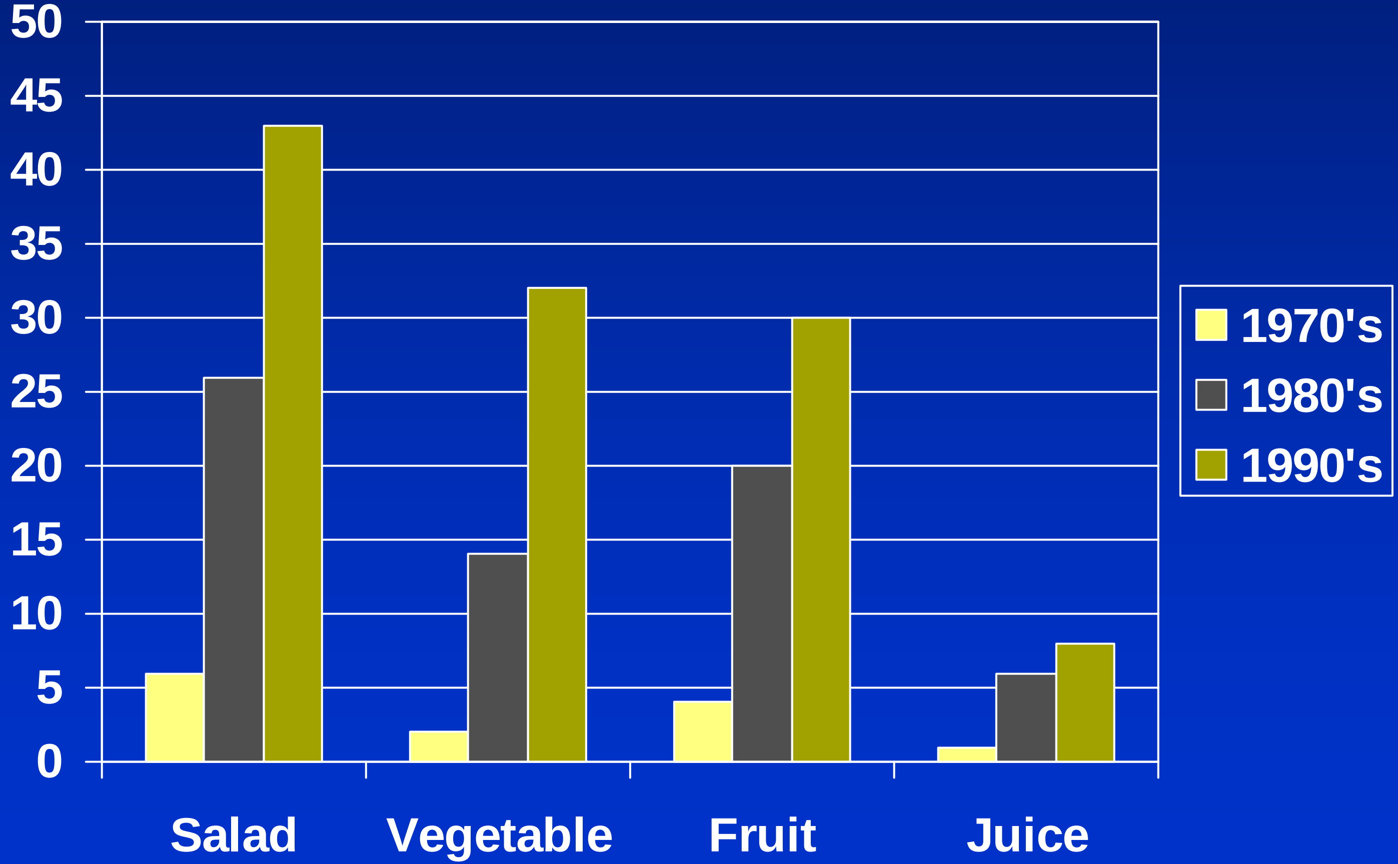
Foodborne outbreaks related to fresh produce, 1973-1997: Specific food vehicles implicated in 190 outbreaks

- Generic or multiple: 105 outbreaks

- Lettuce 25
- Melon 13
- Seed sprouts 11
- Apple or orange juice 11
- Berry 9
- Tomato 3
- Green onion 3
- Carrot 2
- Other 8

88% of outbreaks with one specific vehicle

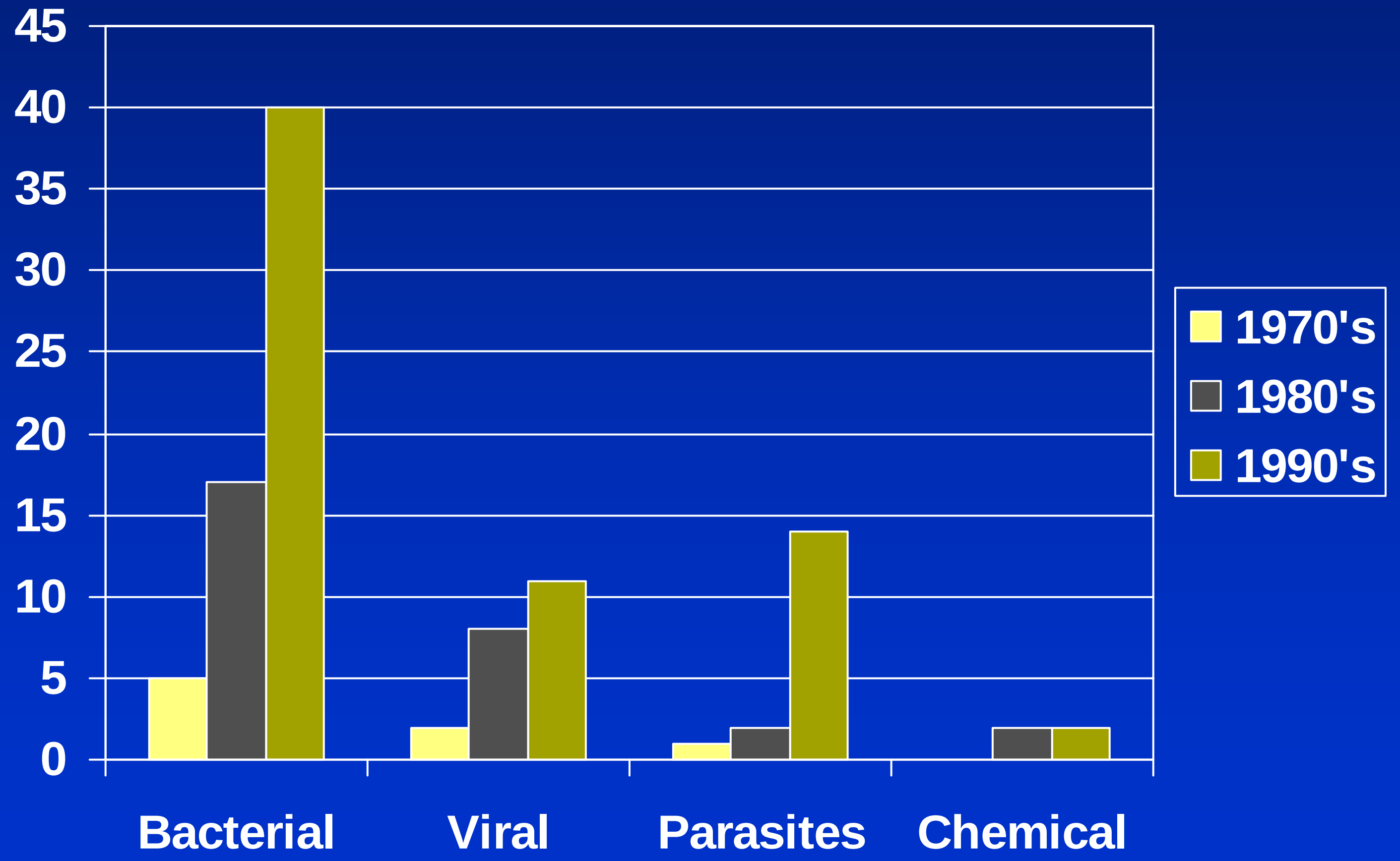
Foodborne outbreaks related to fresh produce, 1973-1997: Time trends in specific vehicle groups



Foodborne outbreaks related to fresh produce, 1973-1997: Pathogens identified in 103 (54%) of outbreaks

Bacterial	**62:**
• *Salmonella*	30
• *E. coli* O157	13
• *Shigella*	10
• *Campylobacter*	4
• Other	5
Viral	**21**
• Hepatitis A	12
• Norovirus	9
Parasite	**16**
• *Cyclospora*	8
• Other	8
Chemicals	**4**

Foodborne outbreaks related to fresh produce, 1973-1997: Trends within pathogen groups



Produce-associated outbreaks reported to CDC in 1998-2002*

- Fresh produce defined in a similar way

- 249 foodborne outbreak linked to fresh produce
  - 10,563 illnesses
  - 311 hospitalizations
  - 5 deaths

- 6% of outbreaks with reported food source
- 13% of outbreak-associated cases

(*Preliminary information)

CDC

Foodborne outbreaks reported to CDC 1998-2002*: Pathogens identified in 179 (72%) of 249 produce associated outbreaks

Bacterial:	**76**
• *Salmonella*	45
• *E. coli* O157	14
• *Shigella*	9
• *Campylobacter*	4
• Other	4
Viral:	**88**
• Norovirus	81
• Other	7
Parasitic:	**6**
Chemical:	**4**

(*Preliminary information)

Foodborne outbreaks reported to CDC 1998-2002*: Pathogens identified in 179 (72%) of 249 produce associated outbreaks

- **Bacterial: 76**
  - *Salmonella* 45
  - *E. coli* O157 14
  - *Shigella* 9
  - *Campylobacter* 4
  - Other 4
- **Viral: 88**
  - Norovirus 81 ← Improved norovirus diagnostics
  - Other 7
- **Parasitic: 6**
- **Chemical: 4**

(*Preliminary information)

Concluded

- Produce is increasingly recognized as a source of foodborne outbreaks, causing more and larger outbreaks
  - Range of pathogens: *Salmonella*, *E. coli* O157, norovirus
  - Produce: Lettuce, melons, tomatoes, sprouts, fruit juices
  - For bacteria, contamination likely before it reaches kitchen

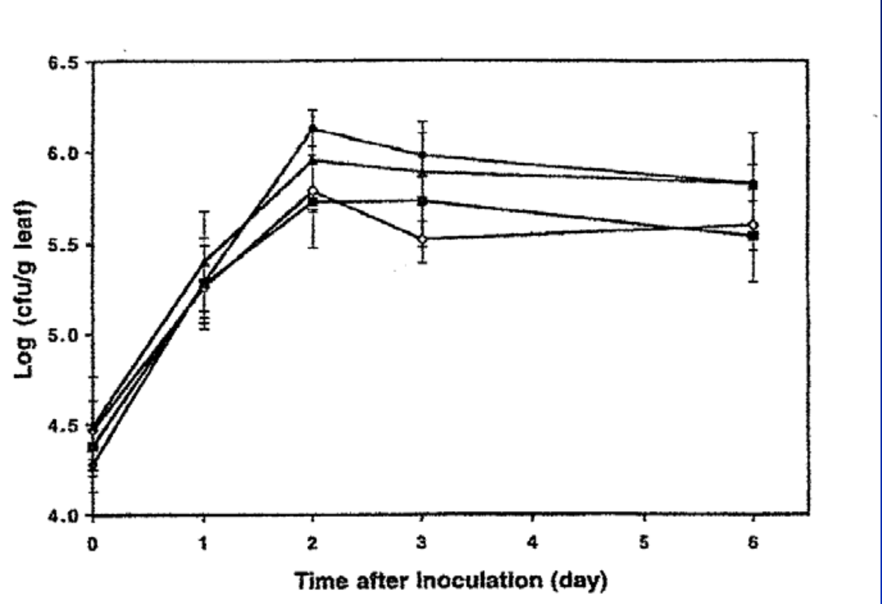
- Contributing factors:
  - Greater consumption of fresh produce
  - Produced in more places, brought more quickly to table
  - Adapting varieties to our desire for sweet

- Not easy to prevent

- Behavior of bacterial pathogens around produce?
  - Food microbiology, plant pathology

Growth and survival on the surface of plant leaves - the phyllosphere

- **Young cilantro plants immersed leaf-first in suspension of *Salmonella* for 2 seconds (10^4 per ml), then held at 24 degrees C and high humidity in greenhouse.**
- **Leaves cultured for next week**
- **Rapidly grew to occupy 80% of total bacterial carrying capacity of leaf surface.**
- **Most along veins and in bruises**
- **4 serotypes tested were equal**

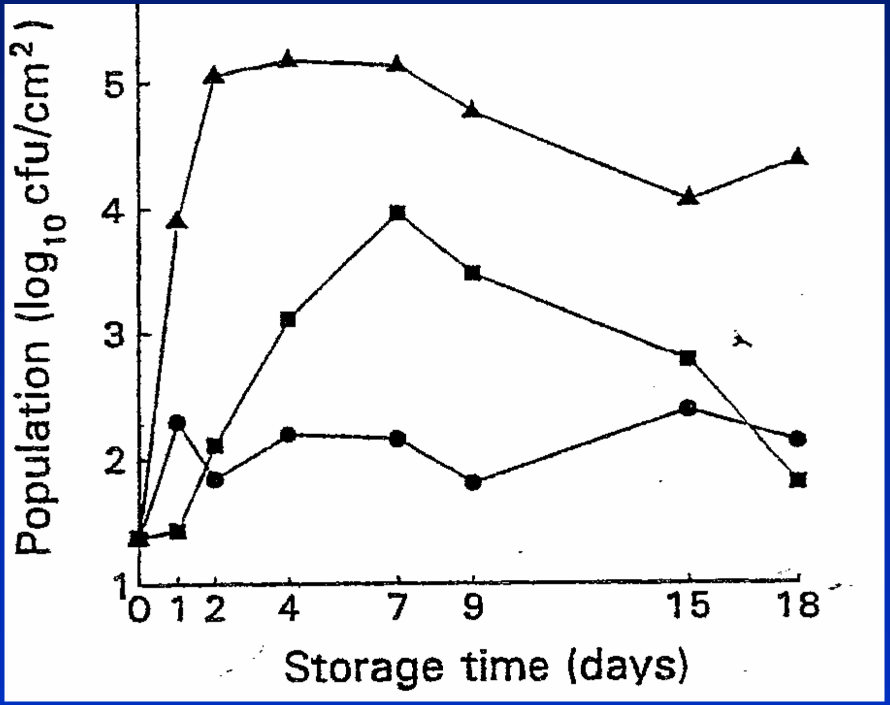


Growth of *Salmonella* Derby, Newport, Enteritidis, Thompson

Brandl MT. Applied Env Microbiol 2002;68:3614-21

Growth and survival on surface of fruits

- **Mature green tomatoes immersed in suspension of *Salmonella* Montevideo for 2 minutes (10^7 per ml), dried, stored at 45-60% relative humidity**
- **Surface rinse of tomatoes at intervals, with quantitative culture**



30

10

20

Growth of *Salmonella* Montevideo at 10, 20, 30 degrees C

Zhuang R-Y. Appl Env Microbiol 1995: 61:2127-2131

CDC

Growth and survival: roots and seeds

- ***Campylobacter jejuni***
  - **Shown for spinach, radishes**
  - **Placed on a leaf, it rapidly succumbs.**
  - **Placed in root zone (rhizosphere), counts slowly decline, but persist for at least 4 weeks**

- ***Salmonella* and *E. coli* O157:H7**
  - **Documented survival on dry seeds for at least 60 days**
  - **Outbreaks traced to alfalfa sprouts sprouted from seeds that are between 1 and 10 years old**
  - **Contamination of the seed likely occurred in the fields.**
  - **Survival probably indefinite**

Brandl. Appl Env Microbiol 2004;70:1182-9

CDC

Internalization

- **The pathogen enters the plant, and then persists**
- **Shown in many plants and many bacterial species**
- **Some unsurprising portals of entry:**
  - **Cuts, open stem scars**
  - **Bruises and “bad spots”**
  - **Capillary action from apple flower calyx to the core**

Burnett Appl Env Microbiol 2000;66:4679-87

Internalization via temperature differential

- Warm fruit in a cold bath --> the internal gasses contract
  - Fruit takes up fluid through the stem scar or calyx
  - Any bacteria in the water, on the surface can be drawn in
- Mangoes into hot water (46 degrees C) for 30 minutes
- Then place them in cool water (22 degrees C) for 15 minutes
  - With 0.1% brilliant blue FD&C
  - Then rinse, dry and cut them open and look for the dye

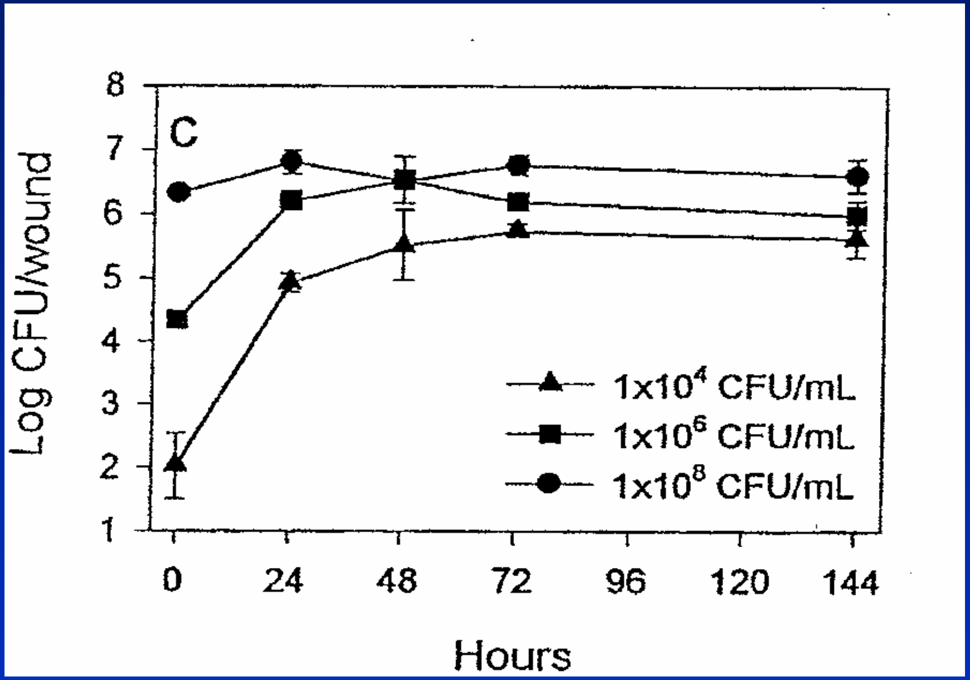
- Demonstrated for:
  - Apples and *E. coli* O157
  - Oranges and *E. coli* O157:H7, *Salmonella*
  - Melons and *Salmonella*
  - Mangoes and *Salmonella*

Penteado. J Food Protection 2004;67:181-184

Internalization via wounds (and fruit flies)

- Punch a cylindrical hole 3mm x 3 mm in an apple.
- Inoculate with broth of E. coli O157:H7
- After a period of time take a 1cc core including the wound, and do quantitative counts

- Fruit-to-fruit transmission via fruit flies demonstrated with non-pathogenic *E. coli*



Quantitative recovery of *E. coli* O157 from wound tissue by time after inoculation

Janisiewicz. Appl Env Microbiol 1999;65:1-5

Internalization via irrigation

- **Irrigate mature lettuce plants with water with *E. coli* O157:H7 was followed by detection of the *E. coli* throughout leaves, stems and roots (10^2-10^3/gram).**

- **Parallel results were obtained with FluoSpheres (10^3/gram)**

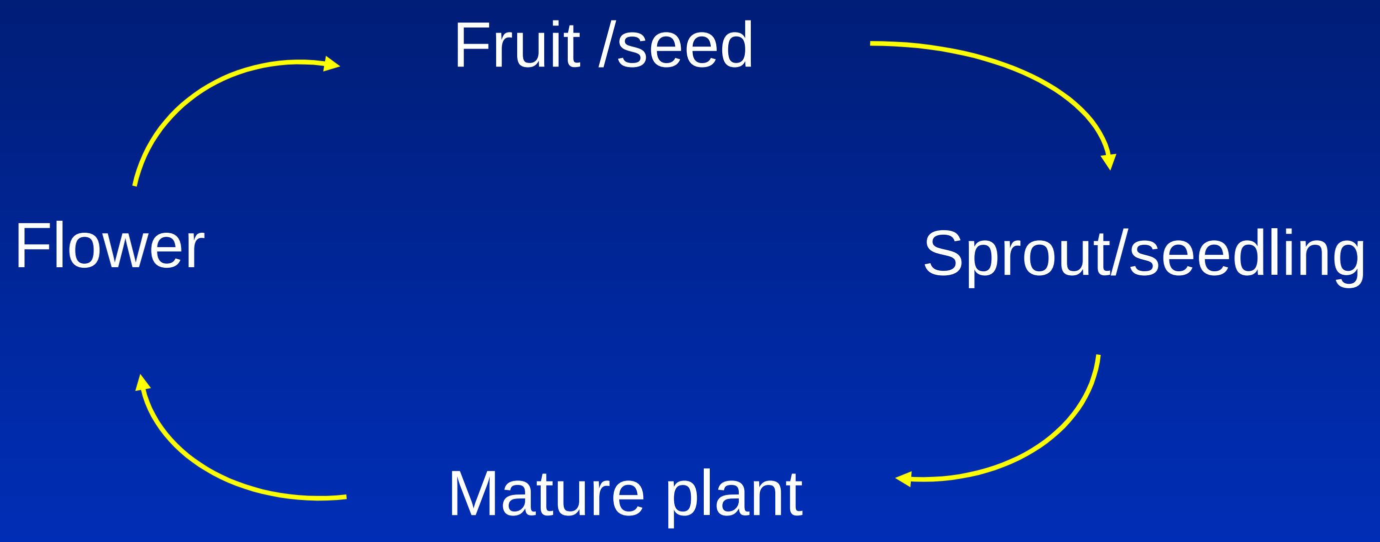
- **Irrigate young tomato plants with mix of *Salmonella* of 5 serovars: Montevideo and Michigan were rapidly absorbed into above ground plant tissues, reaching 10^3 - 10^4/gram. Other serotypes not taken up**

- **Can other bacteria in soil block this uptake?**

Solomon. J Food Protection 2005;68:870-873
Guo. Appl Env Microbiol 2002;68:3639-43
Johannessen. Appl Env Microbiol 2005;71:2221-5

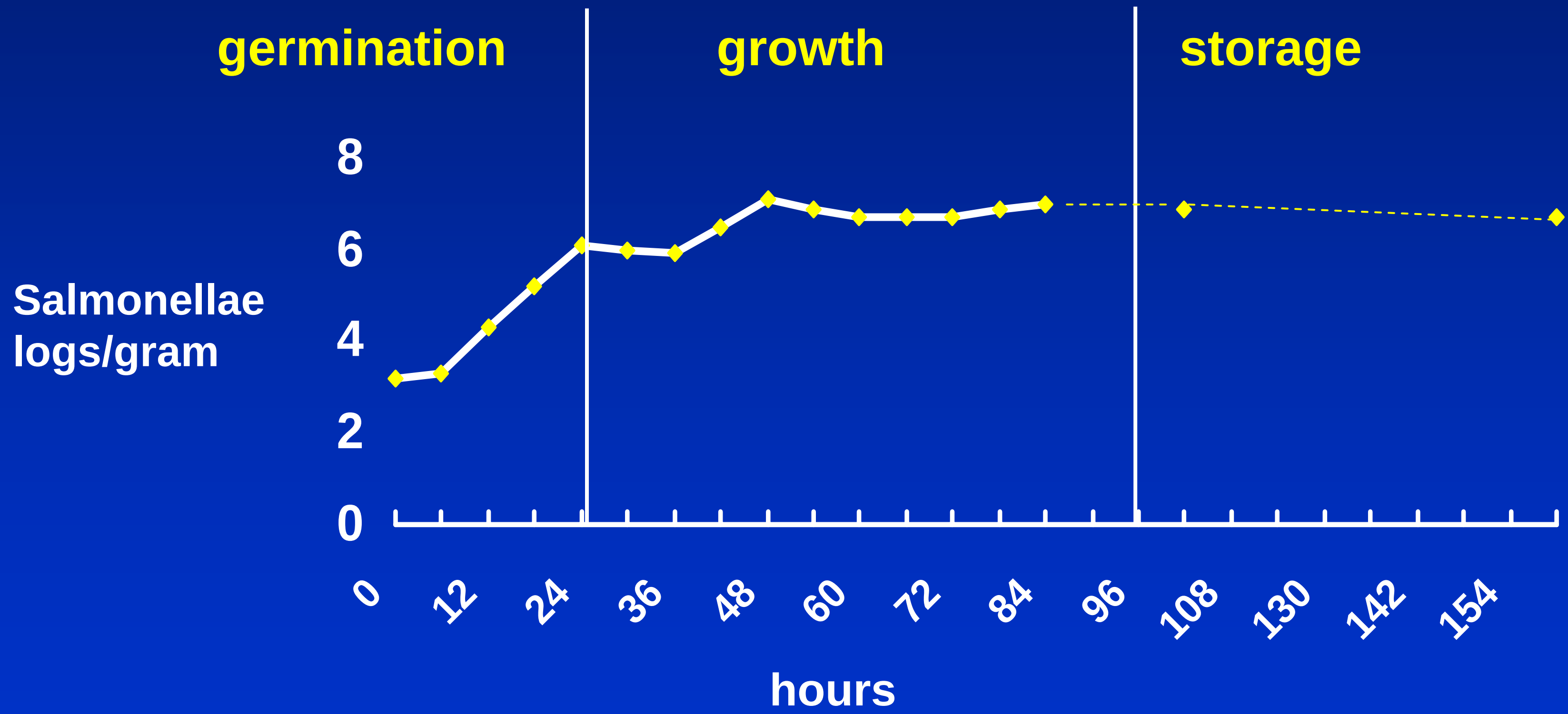
CDC

Riding the life cycle of plants - sprouts



- **Seeds held moist and warm will germinate as sprouts**
- **Bacteria present in the seed will increase and invade the sprouts**
- **Shown for *E. coli* O157, *Salmonella* Typhi, non-typhoidal serotypes**

Rapid growth of *Salmonella* serotype Stanley during sprouting of alfalfa seeds



Jaquette et al, Appl Env Microbiol 1996;62:2212-5

Riding the life cycle of plants - sprouts

- **Studies with strains marked with green fluorescent protein show that *Salmonella* and *E. coli* O157:H7 present in the alfalfa seed coat**
  - **First localize to the roots and root hairs after germination**
  - **Then appear through out the tissues, in the fluid sap within the sprout**
  - **They invade the young plant and colonize all its tissues, without causing it harm**

Charkowski. Appl Env Microbiol 2002;68:3114-20
Itoh. Appl Env Microbiol 1998;64:1532-5

Riding the life cycle of plants - flowers

- Tomato plants 20 cm in height, in flower
- 100 open flowers on eight plants brushed with 5 serovars of *Salmonella*
- 80% aborted
- Eight tomatoes picked when red ripe
- Surface disinfected with 70% ethanol
- Surface and contents of tomato cultured
- 2 of the eight tomatoes yielded *Salmonella*, of three different serotypes. Montevideo and Michigan found in deep tissues

- Suggests that *Salmonella* may enter via pollen tube,at time of fertilization, and persist in the month the new fruit ripens

Guo. Appl Env Micro 2001, 67:4760-64

CDC

Foodborne pathogens and their (other?) reservoirs

- “The idea that some organisms may have the ability to establish themselves and thrive within both plant and warm-blooded animal tissues has received the attention of comparatively few workers. The vast gulf between the two forms of life, in structure, composition and many environmental factors, has seemed to preclude the thought that both could be a favorable host to the same organism.”

- R P Elrod

- Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton.

J Bacteriol 1942:44:633-45

Cross-over bacterial pathogens that infect plants and people (phytoses)

- **Soft rot of tobacco seedlings, onion, cucumbers, potatoes**
  - **Economically significant plant diseases**
  - **1942: bacterial cause clarified by Elrod**
  - **Reproduced these diseases with clinical isolates of *Pseudomonas aeruginosa***
  - **2004: Irrigation challenge studies of sweet basil plants with a clinical Ps a strain that produced biofilm: caused fatal leaf rot**

  - **Clearly a broad host range pathogen**

Walker, Plant Physiology 2004;143:320-31

Cross-over bacterial pathogens that infect plants and people (phytoses)

- **Sour skin bacterial rot of young onions**
  - **1950: Bacterial cause reported by Burkholder, called *Pseudomonas cepacia***
    - **(cepacia means "related to onions")**
  - **1992: Renamed *Burkholderia cepacia***
  - **1997: Van Damme clarified that the onion pathogen is the same biogenovar that causes chronic infections in cystic fibrosis**

Coenye. J Clin Microbiol 2002;39:3427-36

CDC

Cross-over bacterial pathogens that infect plants and people (phytoses)

Pumpkin patch affected by yellow vine disease, 1992

Pumpkin vine cross section
Yellow vine disease

Cross-over bacterial pathogens that infect plants and people (phytoses)

- **Cucurbit yellow vine disease**
  - **Causes yellowing rapid wilting and death of plants**
  - **1988: First detected as an epidemic disease in squash and pumpkin in Midwest**
  - **Subsequently watermelon and cantaloupe**
  - **1997: Gram negative rod identified by EM**
  - **Shown to transmit by the bite of the squash-bug**
  - **Over-winters in the squash bug**

- **2003: Organism identified: *Serratia marcescens* (16sRNA, DNA-DNA hybridization), all CYVD strains are in a recently evolved cluster**

Rascoe. Phytopathology 2003;93:1233-9

Zhang. Phytopathology 2003;93:1240-6

Crossover pathogens and plant models
Pseudomonas aeruginosa

- Mucoid strains from cystic fibrosis patients
  - Tested in wounded alfalfa seedling model
  - Defect in AlgT (sigma factor controlling alginate production) led to loss of invasiveness in seedlings
  - Useful tool to identify factors related to persistence in CF
    - Silo-Suh L. PNAS 2002;99:15699-15704.

- Human clinical isolates studied by ORF deletion
  - Two pathogenicity islands studied
  - Both contained genes for plant and animal virulence
  - Half the genes contributed to virulence in both hosts
    - He J. PNAS 2004;101:2530-2535.

Evolutionary reflections
Biological plausibility

- Enteric bacterial pathogens are well adapted to plants

- They persist on the leaves
- They easily penetrate and then persist in the edible leaves and fruits
- They are readily absorbed from irrigation water
- They may even participate in the seed- flower-fruit cycle
- Most cause no visible harm to the plant
- There are “cross-over pathogens”, pathogenic for both plants and humans

- Why would enteric bacteria, whose home is the gut of vertebrates, have a secret life in plants?

Evolutionary reflections
Colonizing your host's food supply

- We tend to think of enteric bacteria as meat-associated
- However, the meat comes from herbivores
- Herbivores outnumber carnivores

- The capacity to colonize plants herbivore's eat makes sense from an evolutionary view
- The point of edible fruit is to be eaten by a mobile herbivore, who will excrete the seeds somewhere else

- Riding with those seeds, and colonizing next year's growth as it sprouts, gets the bacteria to the next herbivore

Evolutionary reflections
Practical implications

- If enteric bacteria are well adapted to plants, does that affect their ability to cause illness in humans?
- Outbreaks related to plant-derived foods are increasing, and we do not know enough to prevent them.
- Practical questions:
  - Can we reduce the chance of contamination of plant-derived foods by learning more about how such contamination may occur?
  - Can we block the uptake of pathogens into plants?
  - Can we promote the uptake of beneficial bacteria?
  - Can we select new varieties of fruits and vegetables, that make infection less likely?

Evolutionary reflections

- What are the plant host ranges of enteric pathogens?
- What is their survival at the various life stages of plants
- Do they participate in the life cycle of plants?
- How do they interact with other plant-associated bacteria, protozoa, nematodes?

- What genetic traits foster survival in plants, and are they relevant to understanding their behavior in humans?
- Plant models of infection exist, they have few ethical issues, and the reagents are available at the garden supply store.

CDC

Thank you

Foodborne outbreaks reported to CDC 1998-2002*: Spectrum of produce implicated in 249 outbreaks

- Generic or multiple: 144 outbreaks

- Lettuce: 22
- Sprouts: 14
- Juice: 10
- Melon: 9
- Tomato: 8
- Berries: 6
- Cilantro: 4
- Mango: 4
- Other produce items: 17

67% of outbreaks with single vehicle

(*Preliminary information)

CDC