Food Safety and One Health

Foodborne Illness Outbreak Investigation

CONSERVE
Sustainable Water Reuse~Food~Health

CONSERVE is a Center of Excellence supported by the United States Department of Agriculture-National Institute of Food and Agriculture, Grant number 20166800725064.

Agenda

- Introduction to Food Science
- Introduction to Food Safety
- Outbreak Investigation Exercise
- Class Discussion

Educational Objectives

- Characterize the impact of foodborne illness on public health
- Identify factors that contribute to the transmission of pathogens and strategies to minimize risk of disease transmission through food
- Identify investigative stages of foodborne illness outbreak investigations and identify the analytical tools and data utilized for resolution of outbreaks
- Identify various professional roles and regulations associated with assurance of a safe food supply

Food Science Discipline

Encompasses all aspects of …

- Development
- Production
- Processing
- Packaging
- Storage
- Distribution
- Preparation/Handling

While assuring …

- Safety
- Quality
- Stability
- Nutritive Value
- Accessibility
- Affordability
- Sustainability

Foodborne Illness

- Estimated at 48,000,000 per year in the United States (CDC)
  - Gastroenteritis (nausea, vomiting, diarrhea, abdominal pain)
  - Flu-like
  - Other systems can be affected depending on pathogen
    - Neruological (*Clostridium botulinum*)
    - Renal (kidney) (shiga-toxigenic *Escherichia coli* (STEC))
    - Hepatic (liver) (hepatitis A virus)
    - Reproductive (*Listeria monocytogenes*)
  - Severity varies
    - Self-limiting, short duration
    - Hospitalization
    - Long-term sequelae
    - Death
    - Depends on pathogen, host vulnerability, exposure

Foodborne Pathogens

- Etiologies
  - Bacteria (*Salmonella*, pathogenic *E. coli*, *Campylobacter*, *Clostridium*, *Listeria*)
  - Viruses (norovirus, hepatitis A virus)
  - Parasites (*Cryptosporidium*, *Cyclospora*, *Toxoplasma*)
- Many are zoonotic (transmission: human ↔ other animals)
- Transmission: fecal-oral route
- Persistent in food and environmental matrices
- Replication
  - Bacteria – in food or environmental matrices (food storage guidelines)
  - Viruses and Parasites – only in host

Foodborne Illness Outbreak Investigation

- Educational opportunity
  - Problem-solving skills
  - Interdisciplinary connections
  - Roles and strategies
- Investigation stages and data interpretation
  - Epidemiology
  - Laboratory
  - Traceback
  - Environment
  - Prevention

CONSERVE
Food Safety Investigation Exercise

- Collectively work through procedures and issues of foodborne illness outbreak investigation
  - Scenario and group role in investigation
  - Tasks and accompanying clues to generate a 3-digit code
  - Envelope with combination lock – code unlocks lock
  - Group presentations

Groups and Roles

- Group A – Epidemiology
- Group B – Laboratory Investigation
- Group C – Traceback and Recall
- Group D – Environmental Investigation
- Group E – Prevention of Recurrence

Group Tasks

- Activity guidelines
  - Do not alter clues
  - Handle clues gently for reuse
  - Write conclusions on worksheets, blank graphs, questions for class discussion
- Use time wisely
  - Complete tasks correctly to generate a 3-digit code that opens the lock
  - Complete the critical thought questions
  - Prepare to present to the class

Debriefing:

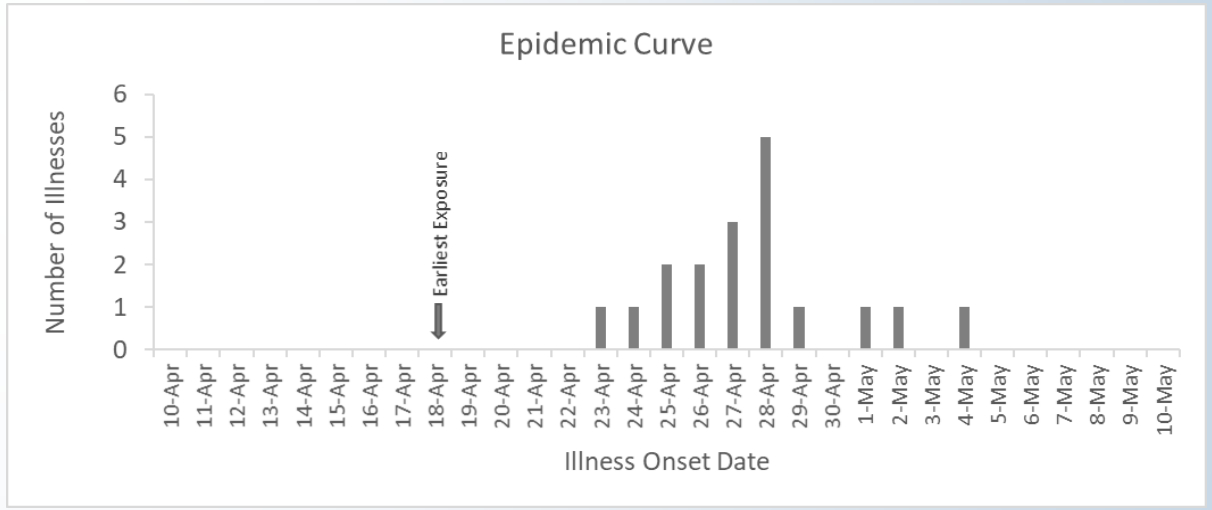
Group Presentations

Group A
Epidemiological Investigation

- Tasks
  - Determine exposure date to illness agent
    - Number of illnesses and illness onset dates
    - Create epidemic curve
  - Determine likely transmission vehicle (contaminated food)
    - Compare foods consumed by ill individuals and non-sick individuals exposed to same foods
    - Calculate odds ratio

Group A
Task 1 – Illness Onset Data and Epidemic Curve

Sick
Female
Age 12 years
Bloody diarrhea, fever, pain
Onset April 27
Condition Poor
Ate Foods # 1 and 2



Conclusion: Earliest exposure date April 18th

Group A
Task 2 – Patient and Control Data

Sick
Female
Age 12 years
Bloody diarrhea, fever, pain
Onset April 27
Condition Poor
Ate Foods # 1 and 2

Not Sick
Female
Age 46 years
Ate Foods # 1 and 2

Odds Ratio
Food Most Associated with Illness

Food	# Ate and Sick	# Ate and Not Sick	# Not Eat and Sick	# Not Eat and Not Sick	Odds Ratio
1	*6*	*8*	*11*	*4*	*1.09*
2	9	7	9	4	0.57
3	15	2	3	10	25

Conclusion: Food #3 Implicated as Transmission Vehicle

Group A
Questions for Further Thought

- Define the term *incubation period*.
- Describe the purpose of calculating the *odds ratio* and the information needed to do so.
- Your group's observations regarding the severity of symptoms as related to patient characteristics.
- Your thoughts on why the date of exposure is important. (How do you think this information is critical to the rest of the investigation)?

Group B
Laboratory Investigation

- Tasks
  - Determine pathogen responsible for illness symptoms
    - Review patient symptoms and compare to food and waterborne pathogen characteristics.
  - Determine time frame for lab results
    - Review the protocol for the suspected disease agent
    - Determine the time to obtain lab results
  - Determine which, if any, of the food sample data matches patient clinical samples.
    - Review data for clinical and food samples
    - Match clinical and food sample isolates

Group B
Task 1 – Etiological Agent

Sick
Female
Age 12 years
Bloody diarrhea, fever, pain
Onset April 27
Condition Poor
Ate Foods # 1 and 2

Select Foodborne and Waterborne Pathogens

	Etiology	Symptoms	Incubation Period	Illness Duration	Foods Associated	Additional Notes
1	*Campylobacter jejuni*	Diarrhea (often bloody), abdominal pain, fever	2 to 10d, usually 2 to 5 d	2 to 10 d	Undercooked poultry, unpasteurized milk, contaminated water	Long-term sequela: Guillain-Barré Syndrome
2	*Clostridium perfringens*	Diarrhea, abdominal cramps	8 to 22 h, usually 10 to 24 h	24 to 48 h	Temperature-abused cooked meats, gravy, beans	Sporeformer, endoenterotoxin
3	*Cyclospora cayetanensis*	Fatigue, protracted diarrhea, often relapsing	1 to 11d, medium: 7d	Weeks to months with relapse	Fresh produce (raspberries, lettuce, basil), contaminated water	Humans only known reservoir, cannot be propagated in laboratory or model animal.
4	*Escherichia coli* (Enterohemorrhagic, (EHEC), shiga-toxin producing (STEC))	Diarrhea (often bloody), abdominal cramps (often severe), low-grade fever, hemolytic uremic syndrome (HUS), kidney failure	1 to 10 d, typically 2 to 5 d	5 to 10 d	Undercooked animal products, raw produce, unpasteurized juice	chronic kidney disease; antibiotic therapy may be contraindicated
5	*Listeria monocytogenes*	Diarrhea, abdominal cramps, fever. If invasive, meningitis, neonatal sepsis, fever	3 to 70 d, usually 4 to 21 d	Variable	Soft cheese, unpasteurized milk, RTE meats, hot dogs	Can cause stillbirth, miscarriage
6	Norovirus	Vomiting, cramps, diarrhea, headache	15 to 77 h, usually 24 to 48 h	12 to 60 hours	Fecally-contaminated foods. Shellfish, fresh produce, RTE handled foods.	Cannot be propagated in laboratory
7	*Salmonella* spp.	Fever, abdominal pain, vomiting, diarrhea	6 to 72 h, typically 18 to 36h	4 to 7 d	Undercooked eggs, poultry, unpasteurized milk or juice, raw produce, chocolate	
8	*Staphylococcus aureus*	Vomiting, diarrhea, abdominal pain	1 to 8h, usually 2 to 4h	24 to 48 h	Improperly refrigerated meats, cream-filled pastries, high protein leftover foods	Intoxication due to preformed toxin
9	*Vibrio parahaemolyticus*	Diarrhea, vomiting, abdominal pain, fever	4 to 96 h, typically 12h	2 to 5 d	Undercooked seafood	

Conclusion: *Escherichia coli* (STEC) etiology

Group B
Task 2 – Time to Lab Results

Protocol for Isolation and Identification of Bacteria

1. Combine test sample with enrichment medium to enhance bacterial growth. Incubate at 37°C for 24 hours.

2. Transfer one ml of enrichment medium to a selective broth medium containing nutrients to enhance growth of suspected bacterial contaminant. Incubate at 37°C for 24 hours.

3. Spread a sample of selective broth medium onto differential agar medium containing nutrients to enhance growth of suspected bacterial contaminant in sample and indicator reagents to aid detection of pathogen among other nonpathogenic microorganisms. Incubate at 37°C for 24 hours.

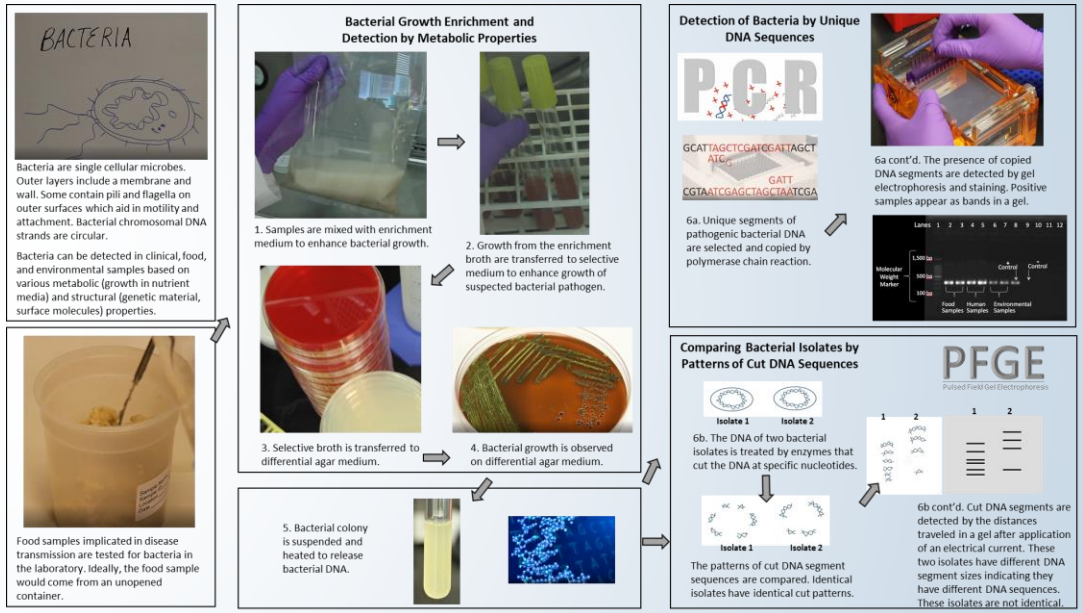
4. Observe microbial growth on agar plates of differential growth medium. Note the appearance of bacterial colonies including colony shape, color, sheen, and the color of surrounding medium for indications of the nutrients in the media that were utilized by the bacteria and the products of bacterial growth. Determine whether the colony appearance is consistent with the suspected etiology for the illness outbreak.

5. Remove a colony from the agar plate, and suspend it in water. Heat the water to 100°C for 10 min to inactivate the bacteria and to release genetic material.

6. Perform analyses of DNA. (Analyses can be done simultaneously requiring approximately 3 hours for each.)

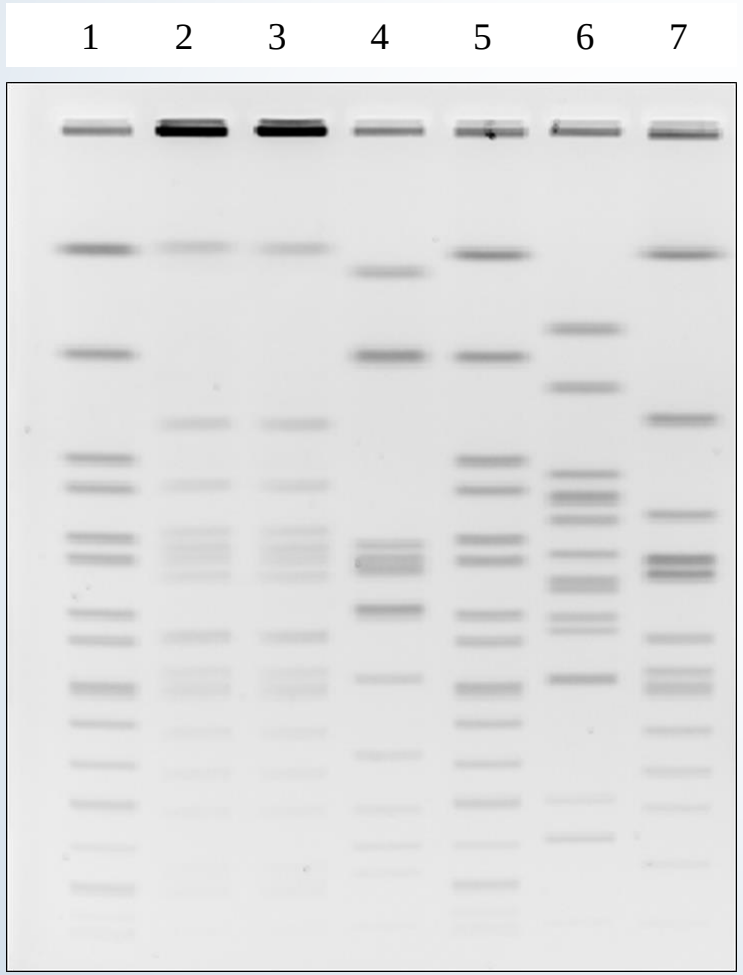
    a. Polymerase Chain Reaction (PCR) - to check for the presence of genes that encode virulence factors (such as toxins) that can cause the illness symptoms.
        i. Suspend DNA in reagents (buffer, nucleotides, and specific sequences of nucleotides that match unique DNA segments).
        ii. Incubate in a thermocycler to select for and make copies of genes that encode virulence factors. A sufficient number of copies is needed for detection.
        iii. Detect copies of DNA that encode for virulence factors (if present) by mixing with fluorescent molecules and measuring fluorescence during incubation, *or* by staining DNA loaded into a gel.

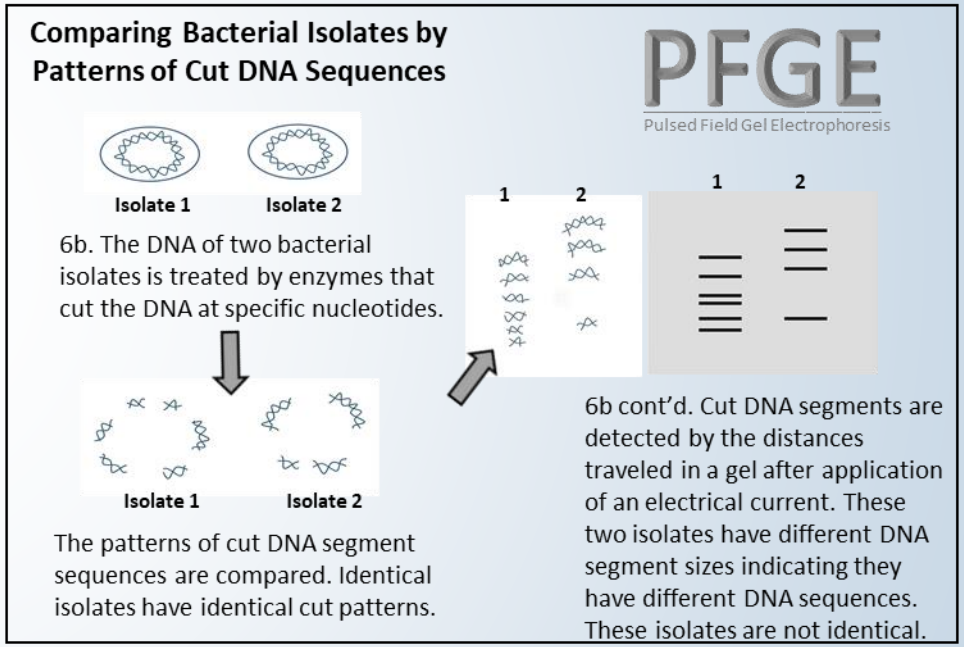
    b. Pulse Field Gel Electrophoresis (PFGE) – to determine if genetic profiles for clinical (stool) isolates and implicated food are indistinguishable.
        i. Suspend DNA in reagents (buffer and enzymes that cut the DNA into pieces at specific sequence locations).
        ii. Load the treated DNA suspension into wells of a gel. Apply an electric current to the gel to make the DNA pieces travel within the gel.
        iii. Stain the gel to detect how far the DNA pieces traveled within the gel; small pieces will travel a greater distance than large pieces.
        iv. Compare the staining patterns for the samples to see which have matching profiles.



Conclusion: 4-day minimum to results

Group B
Task 3 – Match Food and Clinical Samples





Conclusion:
Sample 5 indistinguishable from Reference (lane 1)

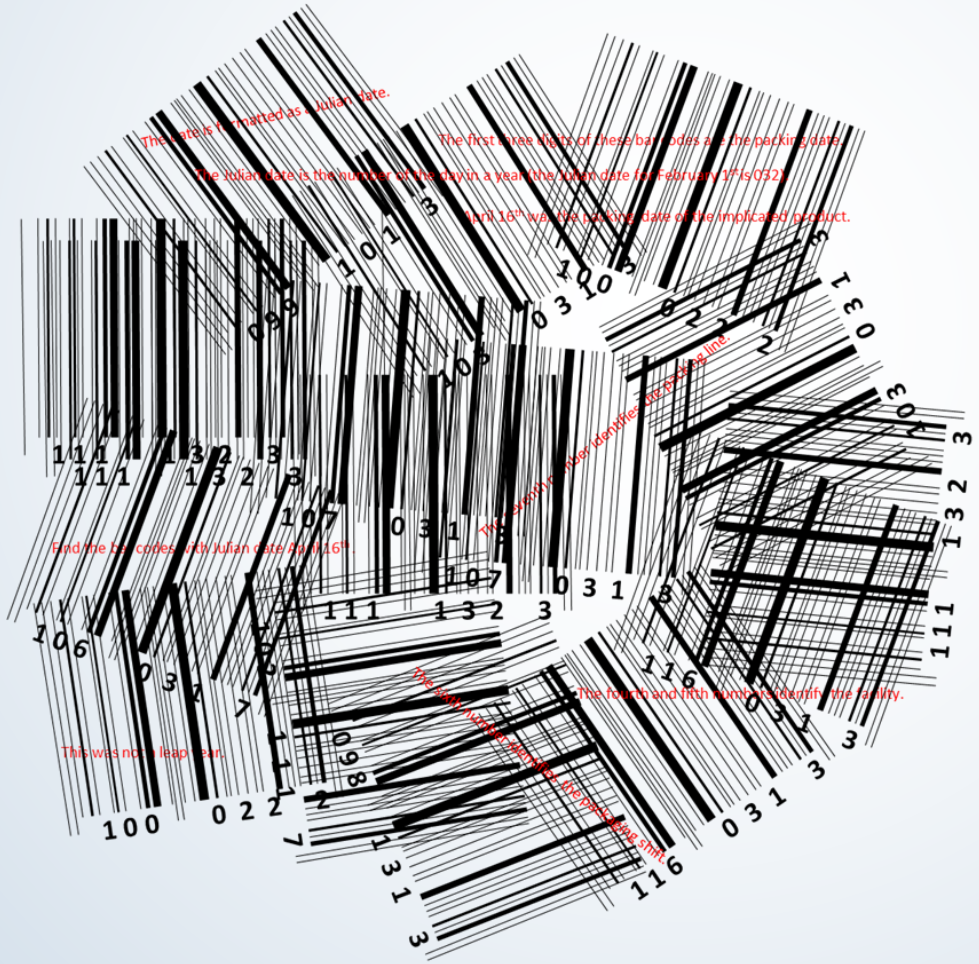
Group B
Questions for Further Thought

- *E. coli* STEC symptoms and transmission vehicles
- Your thoughts on if a food sample does not test positive for the same disease agent identified in stool samples, does it *guarantee* the food product was *not* associated with the outbreak?

Group C
Trace Implicated Product back to Source and Determine Extent of Distribution

- Tasks
  - Begin traceback to product source by interpretation of product label codes
    - Determine the single-digit facility number associated with the implicated product.
  - Trace product back to source through distribution records
    - Determine the product source (producer #) associated with the implicated product
  - Determine breadth of distribution of implicated product to support recall efforts
    - Determine how many states to which implicated product was distributed.

Group C
Task 1 – Begin Traceback – Interpret Product Codes



1 0 6 0 3 1 7

Digits 1, 2, 3: Julian date 106 (April 16th)
Digits 4, 5: Facility Number (03)
Digit 6: Production Shift (1)
Digit 7: Production Line (7)

Group C
Task 2 - Trace Product Source

Packing Facility Records for Sources of Products

Date	Shift	Production Line	Product Source (Producer #)
April 15	2	7	3
April 15	2	8	3
April 15	2	9	3, 4
April 16	1	1	1
April 16	1	2	1
April 16	1	3	2
April 16	1	4	2, 3
April 16	1	5	3
April 16	1	6	3
April 16	1	7	4
April 16	1	8	4
April 16	1	9	5
April 16	2	1	6
April 16	2	2	6
April 16	2	3	7, 8
April 16	2	4	9
April 16	2	5	1
April 16	2	6	2
April 16	2	7	2
April 16	2	8	3
April 16	2	9	3
April 17	1	1	1
April 17	1	2	3
April 17	1	3	3

Matches bar code →

← Note: same source on Production line 8

Conclusions:

- Implicated product sourced from Producer #4
- Other bar codes affected (Line 8)

Group C
Task 3 - Determine distribution of implicated product

Packing House Distribution Records

Date	Shift	Production Line	Product Source (Producer #)	Wholesale	Retail
April 15	2	7	3	AZ, CA, NM	AZ, CA, NM
April 15	2	8	3	AZ, CA, NM	AZ, CA, NM
April 15	2	9	3, 4	AZ, CA, NM	AZ, CA, NM
April 16	1	1	1	AZ, CA, NM	AZ, CA, NM
April 16	1	2	1	AZ, CA, NM	AZ, CA, NM
April 16	1	3	2	AZ, CA, NM	AZ, CA, NM
April 16	1	4	2, 3	AZ, CA, NM	AZ, CA, NM
April 16	1	5	3	AZ, CA, NM	AZ, CA, NM
April 16	1	6	3	AZ, CA, NM	AZ, CA, NM
April 16	1	7	4	AZ, CA, NM	AZ, CA, NM
April 16	1	8	4	AZ, CA, DE, MD, NJ, NM, NY, PA	AZ, CA, DE, MD, NJ, NM, NY, PA
April 16	1	9	5	AZ, CA, DE, MD, NJ, NM, NY, PA	AZ, CA, DE, MD, NJ, NM, NY, PA
April 16	2	1	6	AZ, CA, DE, MD, NJ, NM, NY, PA	AZ, CA, DE, MD, NJ, NM, NY, PA
April 16	2	2	6	AZ, CA, DE, MD, NJ, NM, NY, PA	AZ, CA, DE, MD, NJ, NM, NY, PA
April 16	2	3	7, 8	AZ, CA, DE, MD, NJ, NM, NY, PA	AZ, CA, DE, MD, NJ, NM, NY, PA
April 16	2	4	9	AZ, CA, DE, MD, NJ, NM, NY, PA	AZ, CA, DE, MD, NJ, NM, NY, PA
April 16	2	5	1	AZ, CA, DE, MD, NJ, NM, NY, PA	AZ, CA, DE, MD, NJ, NM, NY, PA
April 16	2	6	2	AZ, CA, DE, MD, NJ, NM, NY, PA	AZ, CA, DE, MD, NJ, NM, NY, PA
April 16	2	7	2	AZ, CA, DE, MD, NJ, NM, NY, PA	AZ, CA, DE, MD, NJ, NM, NY, PA
April 16	2	8	3	DE, MD, NJ, NY, PA	DE, MD, NJ, NY, PA
April 16	2	9	3	DE, MD, NJ, NY, PA	DE, MD, NJ, NY, PA

Matches bar code

Note:
Same source on Production line 8

Conclusion:
Implicated product distributed to 8 states

Group C
Questions for Further Thought

- Your thoughts on how traceability software could impact investigations and product recalls.

- Your thoughts on what measures would be needed to notify and protect the public from a contaminated product in commerce with a long shelf life or as a common ingredient in multiple products.

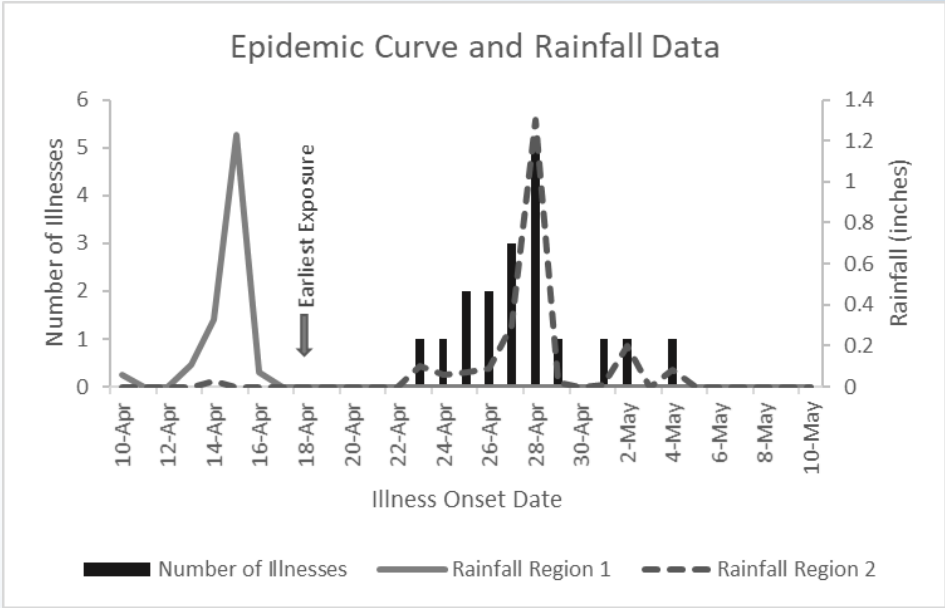
Group D
Environmental Investigation

- Tasks
  - To evaluate the production environmental conditions (heavy rainfall) that may have contributed to contamination of the implicated food product.
  - To evaluate the production environment and practices (water source) that may have contributed to contamination of the implicated food product.
  - Determine the risk for foodborne disease transmission by irrigation method and commodity.

Group D
Task 1 – Rainfall potential impact on transmission

Rainfall data from two regions

Rainfall data overlaid on epidemic curve



Conclusion:
Rainfall in Region 1 prior to harvest potential impact on pathogen transmission

Group D
Task 2 – Environmental risk factors

Conclusion:
Water sourced for Field #4 at greatest risk from environmental transmission of pathogens

Group D
Task 3 – Pathogen transmission risk cont'd.

Irrigation Method

Irrigation Method #1
Drip Irrigation

Irrigation Method #2
Overhead Irrigation

Right: "Crop Irrigation" by aqua.mech is licensed under CC BY 2.0

Commodity

Produce Item #1
Apple

Produce Item #2
Carrot

Produce Item #3
Lettuce

Produce Item #4
Potato

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"File:Romaine lettuce.jpg" by Rainer Zenz is licensed under CC BY-SA 3.0
"Potatoes" by 16:9clue is licensed under CC BY 2.0

Conclusions:

- Overhead irrigation greater risk for pathogen spread to edible portion of plant if water is contaminated
- Produce <u>not</u> consumed raw (potato) lowest risk as pathogen transmission vehicle

Group D
Questions for Further Thought

- The role of environmental waters for potential transmission of microorganisms to food crops

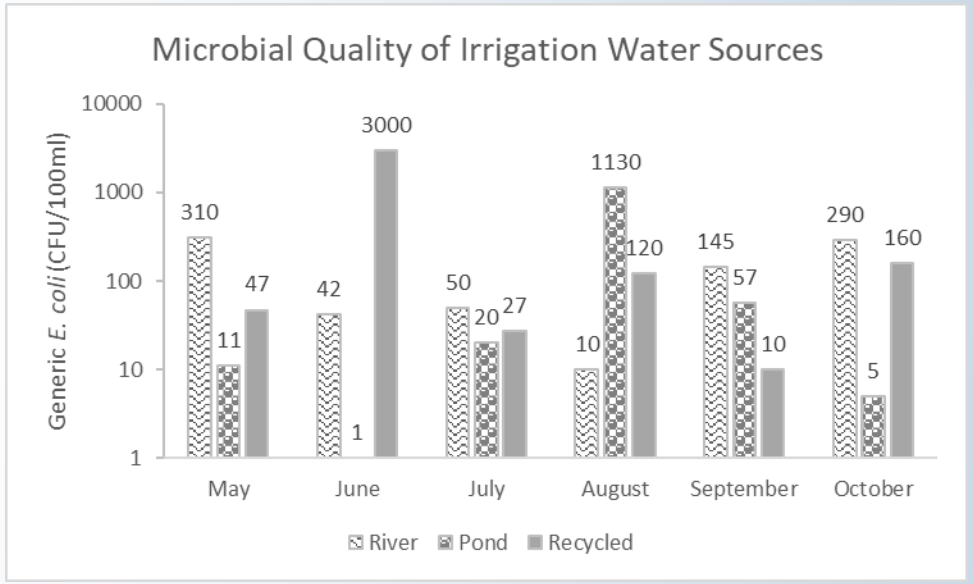
- Your thoughts on what actions a grower could take to minimize risk of contamination to harvested crops if there were a heavy rain event shortly before harvest

Group E
Prevention of Recurrence

- Tasks
  - Evaluate the potential microbial risk of various water sources for irrigation of edible crops to address water scarcity issues
  - Determine whether water sources meet regulatory standards of Produce Safety Rule of Food Safety Modernization Act (FSMA)
  - Evaluate potential treatment methods to improve the microbiological quality of irrigation water

Group E
Task 1 – Water quality (generic *E. coli*) variability

Water Quality Data
Generic *E. coli*
(Not pathogen counts)

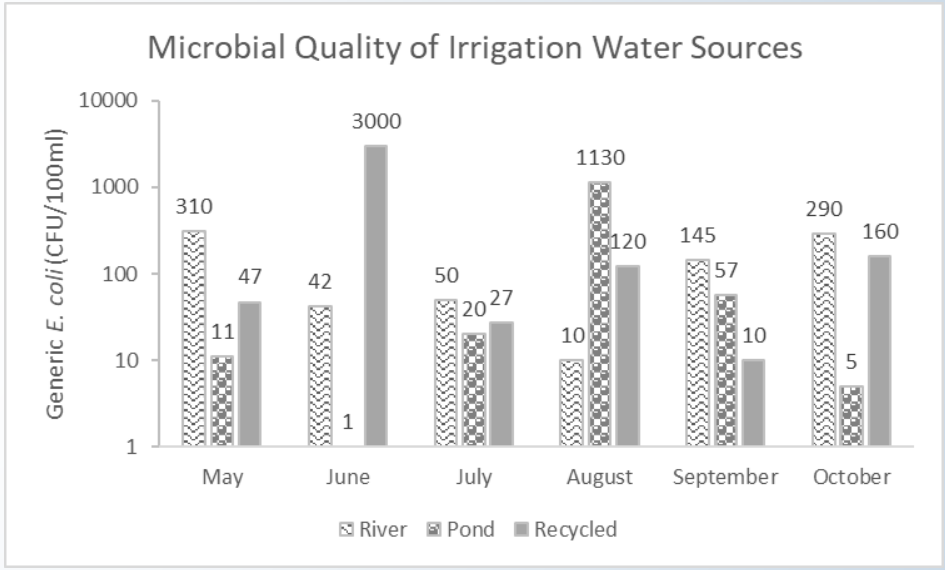


Conclusion:
Recycled water has the greatest variability in detectable generic *E. coli* as indicator of quality

Group E
Task 2 – Water source and regulatory compliance

The Food Safety Modernization Act (FSMA) was signed into law in 2011 and includes the **Produce Safety Rule**. One requirement of the Produce Safety Rule is that water quality used for irrigation of food crops is to be monitored over time in consideration for variability due to one-time events such as heavy rainfall. While somewhat an oversimplification of the rule, **the water microbial standards for growing foods** (other than sprouts) **call for no more than an average of 126 colony-forming units (CFU) of generic *E. coli* (as an indicator of fecal contamination) in 100 ml of water** (https://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334114.htm#key).

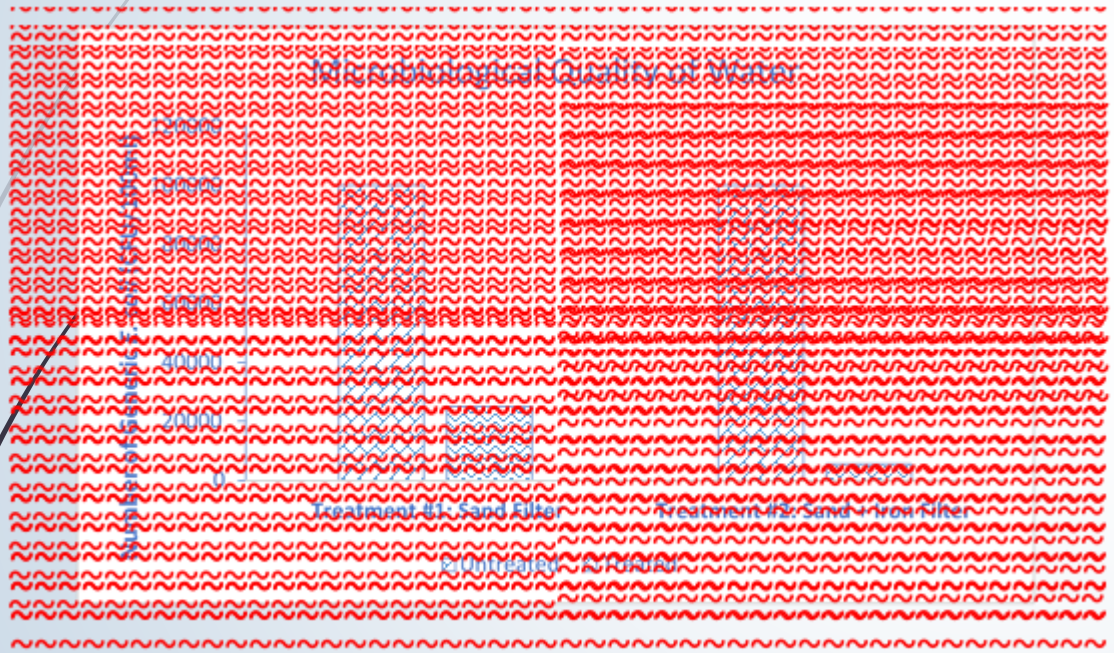
Which, if any, of the water samples meets this standard?



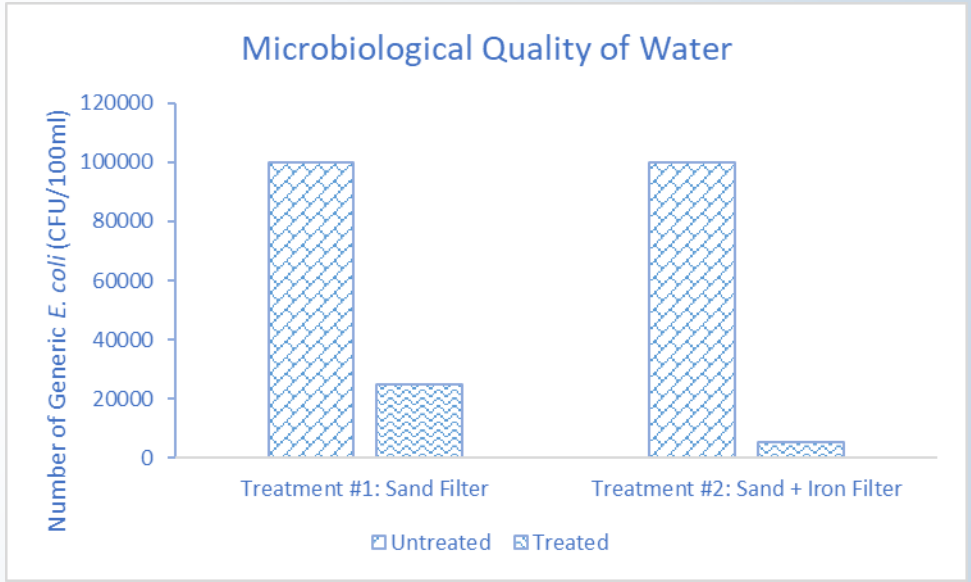
Conclusion:
None of the untreated water sources meet the regulatory standards of the U. S. FDA Produce Safety Rule for irrigation of edible crops. Water treatment is needed to remove microorganisms.

Group E
Task 3 – Treatment efficacy for bacteria removal

Clue provided with 'decoder'



Water Treatment Data



Conclusion:
Filter with both sand and iron removes more generic *E. coli* than filter with just sand

Group E
Questions for Further Thought

- Examples of water sources investigated for irrigation of edible crops as alternatives to groundwater and emerging water remediation methods to address water scarcity issues.

- Your thoughts on what contaminants other than microorganisms that might be present in environmental waters as result of human activity

Summary

- Foodborne and waterborne disease
  - Zoonotic
  - Environmental (among other contamination routes)
  - One Health connections – human, animal, plant, environment
- Investigation
  - Epidemiology
  - Laboratory
  - Traceback and Recall
  - Water as a pathogen transmission vehicle (among others)
  - Alternative water sources (to address water scarcity)
  - Water treatment technologies (prevention of recurrence)
- Professional scientific roles
  - Public health
  - Laboratory
  - Regulatory
  - Industry
  - Research

This material is based upon work supported in part by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2016-68007-25064.