



Fresh Produce and Pathogenic Microbiology

Stephanie Smith, Ph. D.
Consumer Food Safety Specialist
Washington State University
March 9, 2020



The Cultivating Success™ Program
is a partnership of...



University of Idaho
Extension



Food Systems

WASHINGTON STATE UNIVERSITY

Visit us at www.cultivatingsuccess.org

Today's Presenter



Stephanie Smith, Ph. D.
Consumer Food Safety Specialist
Washington State University
steph.smith@wsu.edu



Colette DePhelps, moderator
Area Extension Educator in Community Food Systems, Northern District
University of Idaho Extension
cdephelps@uidaho.edu



Fresh Produce and Pathogenic Microbiology



Overview

- Background on Foodborne Illness
- Sources of Contamination for Fresh Produce
- Some Pathogens Associated with Fresh Produce
- Steps to Prevent Contamination



Foodborne Illness



Fresh Produce Contaminated with Food Pathogens..

Can cause a **foodborne illness** with the most common symptoms being:

- Vomiting
- Diarrhea
- Fever
- Chills
- Abdominal pain

Severe Symptoms can include:

- Reactive arthritis
- Organ failure
- Brain and nerve damage

Most symptoms are self resolving but can cause hospitalization and death.

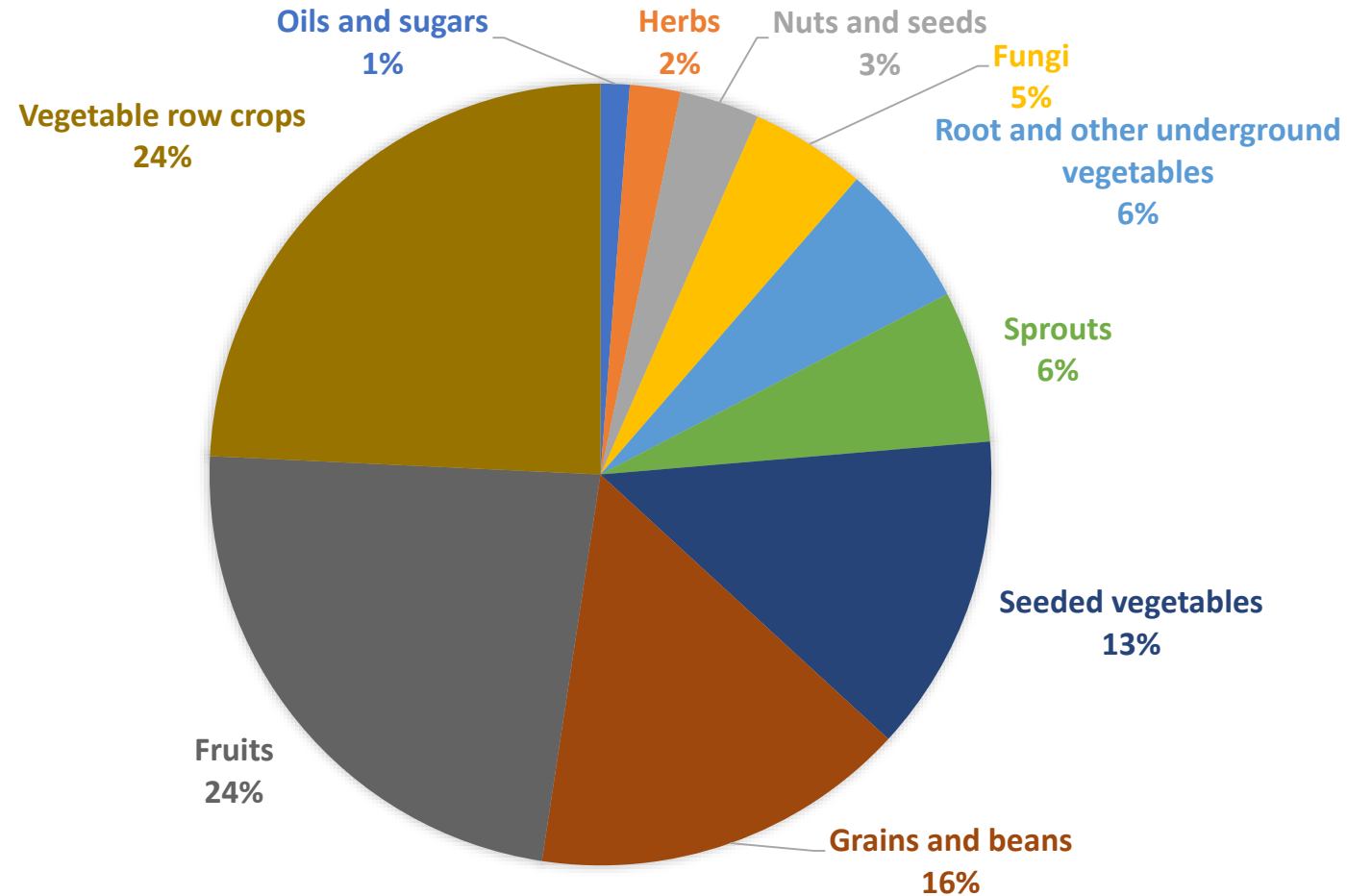
The Stats on Foodborne Illness

“Each year an estimated 48 million Americans are stricken ill as the result of one food-borne pathogen or another—listeria, *E. coli*, salmonella, and even a number of stowaway viruses are among the culprits.”

“A 2015 study by Robert Scharff, an associate professor at Ohio State University, estimates the annual cost of medical treatment, lost productivity, and illness-related mortality at \$55.5 billion.”



Plant-Associated Outbreaks in United States and Puerto Rico, 2009-2015



Dewey-Mattia et al., 2018

Selected Fresh Produce-Linked Outbreaks and their Etiological Agents

- Romaine lettuce - *E. coli* O157:H7 (2018, 2019)
- Leafy greens - *E. coli* O157:H7 (2017)
- Cucumbers - *Salmonella* Poona (2015), *Salmonella* Newport (2014), *Salmonella* Saintpaul (2013)
- Cilantro - *Cyclospora* (2014)
- Mangoes - *Salmonella* Braenderup (2012)
- Cantaloupes - *Salmonella* Typhimurium and Newport (2012), *Salmonella* Panama (2011), *Listeria monocytogenes* (2011)

<https://www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html>



How Does Produce Become Contaminated?

Source of Contamination for Fresh Produce



- Inadequate worker health and hygiene
- Soil and improper use of biological soil amendments
- Contaminated agricultural water (production and postharvest water)
- Wildlife and domestic animals
- Improper postharvest handling and sanitation

There is no "kill step" for fresh produce, freezing or refrigeration does not kill pathogens, and once contaminated, it is nearly impossible to remove quickly.



Worker Health and Hygiene

- Inadequate worker hygiene (unwashed or not properly washed hands, contaminated clothing and tools);
- Worker health issues (unreported sickness, injuries resulting in spreading blood or bodily fluids on produce)
- Most common pathogens transmitted by food contaminated by employees: Hepatitis A, *Salmonella typhi*, *Shigella* spp., Norovirus, *Staphylococcus aureus*, *Streptococcus pyogenes*



Soil and Biological Soil Amendments

- Soil (natural reservoir of both beneficial and pathogenic microorganisms);
- Improper application of manure (type of produce, time of application);
- Improper composting (not scientifically validated methods; not following the composting procedure)



Wildlife and Domestic Animals

- Feces contain human pathogens;
- Feces are spread by animals
- Harvesting visibly contaminated fresh produce (e.g. with birds' droppings)
- Harvesting dropped fresh produce from the ground (might go in contact with feces on the ground)



Agricultural Water – Production

- Contamination with wildlife and domesticated animal's feces
- Contamination with manure, runoff from livestock farms, or urban or environmental sources such
- Waste water discharge, septic tank leakage



Agricultural Water - Postharvest

- Use of inappropriate water (e.g. surface water) source during postharvest activities such as rinsing produce
- Contaminated harvest containers, equipment (packaging tables, conveyer belts, tools),



Postharvest Handling and Sanitation

- Inadequate worker hygiene (unwashed or not properly washed hands, contaminated clothing and tools)
- Worker health issues (unreported sickness, injuries resulting in spreading blood or bodily fluids on produce)
- Lack of cleaning and sanitation or these activities are not executed properly
- Pests (carrying human pathogens)



Most Common Fresh Produce Food Pathogens

Pathogenic Microorganisms in Fresh Produce

Bacteria – e.g.
Escherichia coli,
Salmonella, *Shigella*,
Listeria monocytogenes

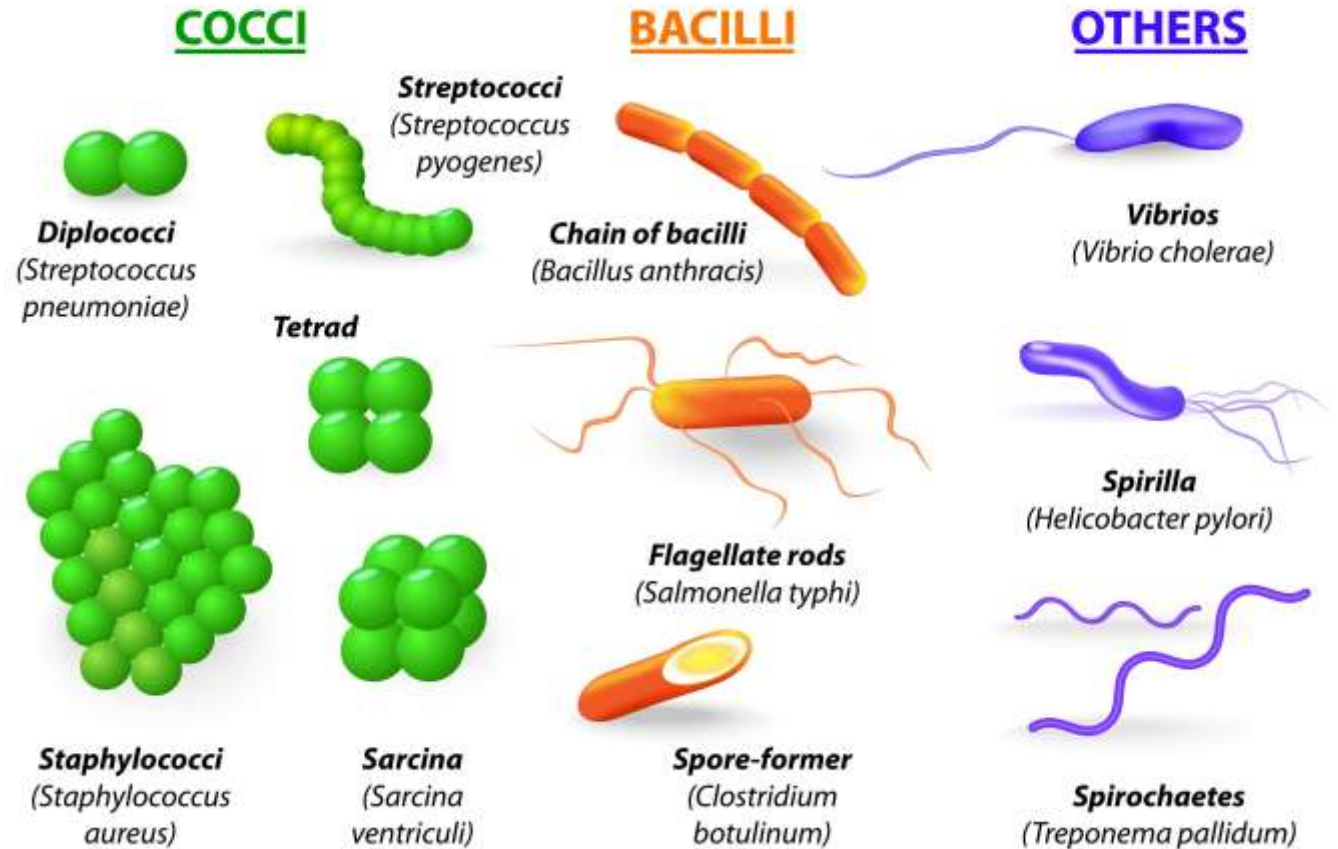
Viruses – e.g. Norovirus,
Hepatitis A

Parasites – *Toxoplasma*
gondii, *Cyclospora*,
Giardia

Bacteria

- Single-cell microscopic size organisms
- Classified-based on the cell shape, or cell-wall structure (Gram positive or negative); taxonomy based on 16S rRNA sequences
- Live in diverse environments, e.g. soil, water, plants, humans
- Can be beneficial; kombucha, yogurt, sauerkraut are products of fermentation

SHAPES OF BACTERIA



Bacterial Growth Requirements – FAT TOM

Food – various nutrients

Acidty - pH 4.6-8.0

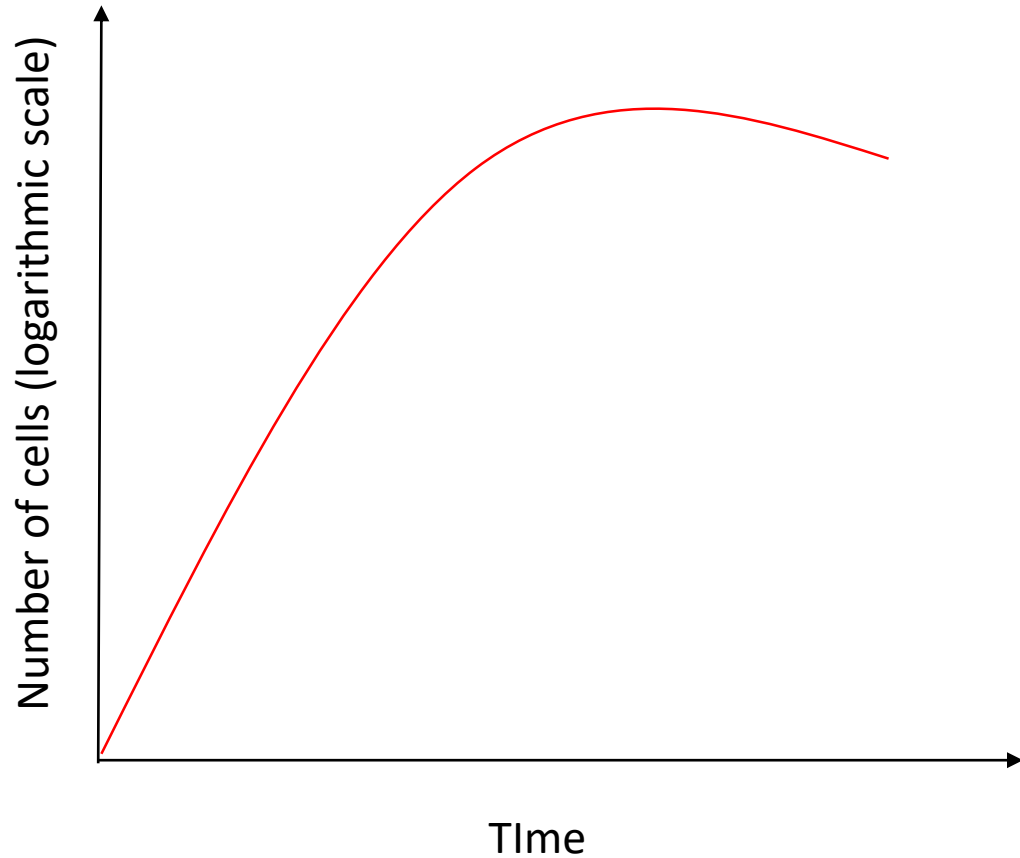
Time – some microorganisms can grow and divide every 20 minutes!

Temperature – organisms can grow between 41°-135°F

Oxygen – some need oxygen but some don't

Moisture - Water activity (a_w) – bacteria can multiply when it is above 0.86

Bacterial Growth

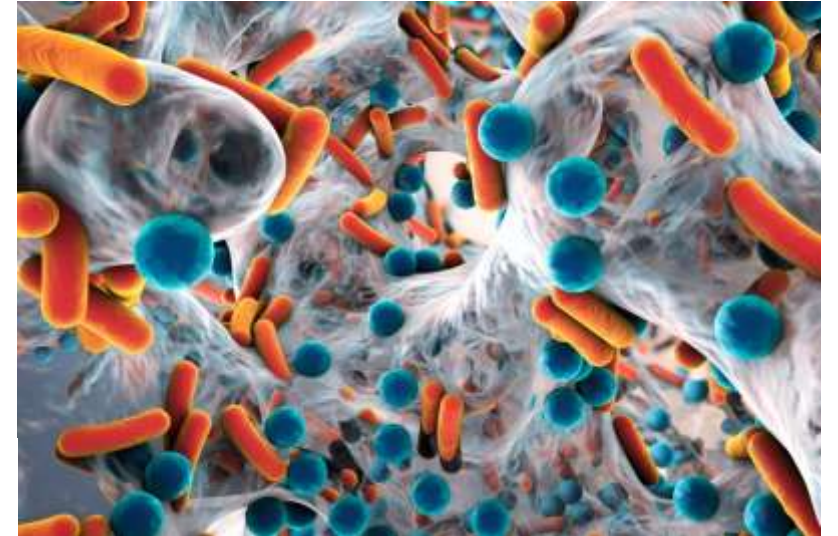


Time	Number of cells
0 min	1
20 min	2
40 min	4
1 hr	8
2 hrs	16
3 hrs	512
4 hrs	4,096
5 hrs	32,768
6 hrs	262,144
7 hrs	2,097,152
8 hrs	16,777,216

How Many Bacteria Does it Take to Cause Illness?

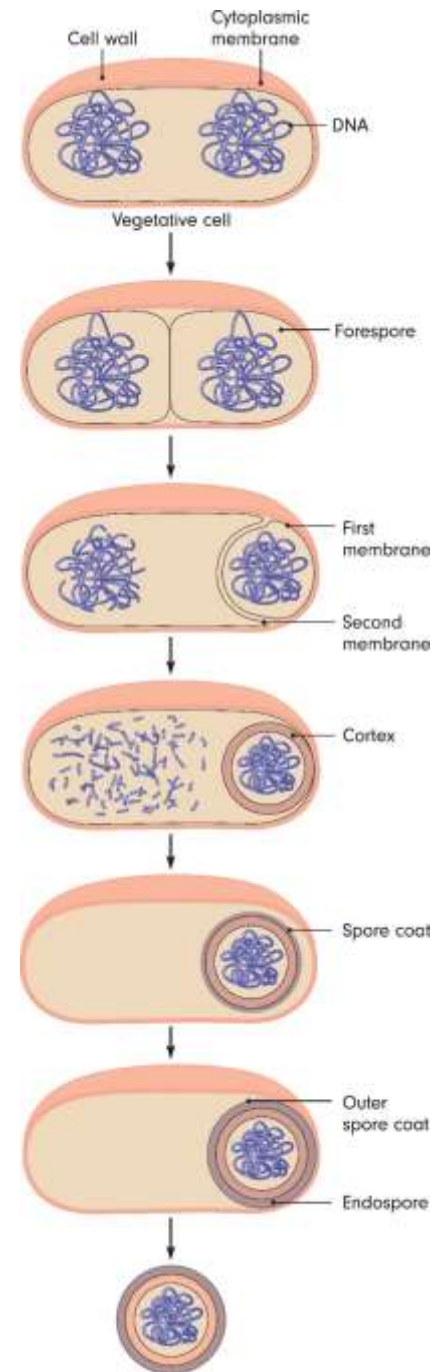
Organism	Infectious Dose
<i>E. coli</i> O157:H7	10-100
<i>Campylobacter</i>	< 500
<i>Listeria</i>	<1000
<i>Bacillus cereus</i>	>100,000

* 500 bacteria can be present in one drop of meat juice



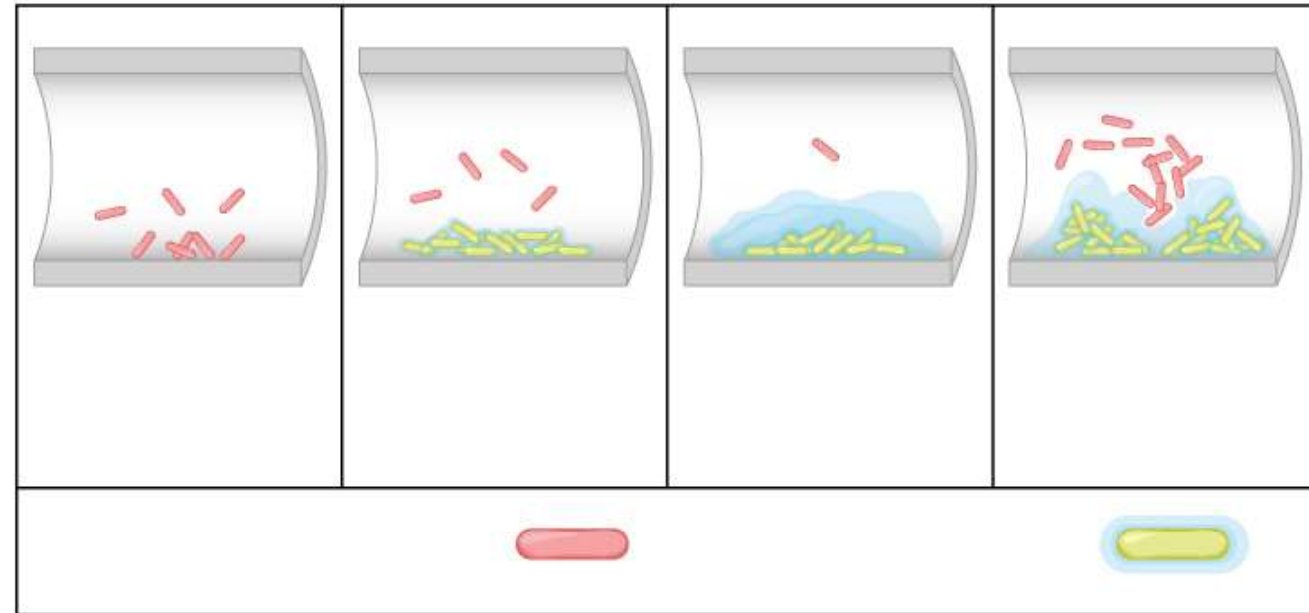
Bacterial Spore Formers

- Some bacteria are spore-formers (e.g. *Bacillus cereus*, *Clostridium perfringens*, *Clostridium botulinum*);
- Vegetative cells enter sporulation process when they encounter unfavorable environmental conditions;
- Spores can withstand harsher environmental conditions e.g. elevated temperature, lack of oxygen, pH variations.



Bacterial Biofilms

- Assemblages of bacterial cells surrounded by extracellular polymeric matrix
- Free (planktonic) cells attach to surface, and this process is complex and depends surface and cell properties and surrounding environment conditions
- Can colonize: water, pipelines, produce contact surfaces, gloves, packaging materials.



Salmonella spp.

- Motile, Gram-negative, non-sporeforming, biofilm producing rods
- *S. enterica* and *S. bongori* are the cause of foodborne illness in humans
- *Salmonella* is divided into serotypes based on the antigenic properties of their surface and flagella; there are currently over 2,500 serotypes named with common names (not italicized)
- Can cause two types of illness:
 - **nontyphoidal salmonellosis** – nausea, vomiting, diarrhea, headache; self-limiting
 - **typhoidal fever** - more serious with higher mortalities; high fever, gastrointestinal symptoms, headache
- **Produce contamination source:** commonly present in nature: soil, intestines of wildlife, livestock (birds); by cross-contamination of production and postharvest water, inadequate worker hygiene
- **Commonly contaminated fresh produce but not limited to:** tomatoes, peppers, cantaloupes, and other



Figure 1: *Salmonella* serotype Typhi bacteria – computer generated image based on scanning electron microscopic photograph.

Shigella spp.

- Non-motile, Gram-negative, non-sporeforming, rods
- Humans are the only hosts
- Includes *S. sonnei*, *S. boydii*, *S. flexneri*, *S. dysenteriae*
- Some strains produce enterotoxins and Shiga toxins
- Cause **shigellosis** with symptoms such as diarrhea (from watery to acute, bloody and life threatening)
- Heat-sensitive and do not have complex nutrient requirements but survive low pH
- **Fresh produce contamination source:** cross-contamination of production and postharvest water with feces, inadequate worker hygiene
- **Commonly contaminated fresh produce but not limited to:** lettuce

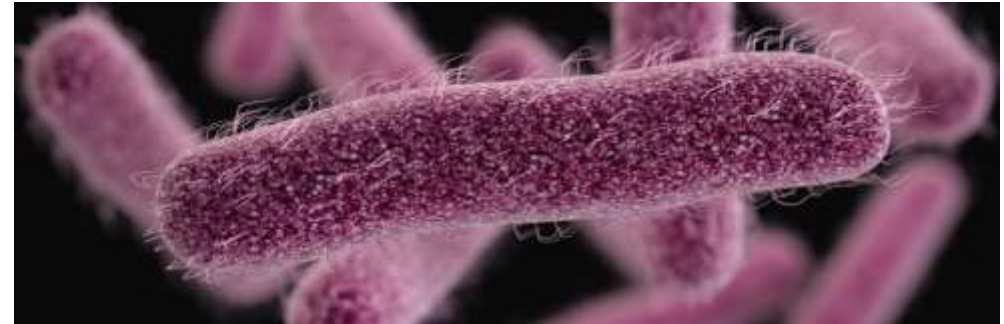


Figure 2. *Shigella* bacteria – computer generated image based on scanning electron microscopic photograph.

Escherichia coli (STEC)

- Motile, Gram-negative, non-sporeforming, biofilm producing rods
- Constitutes a normal microflora of human and animal intestines
- Small group of *E. coli* is pathogenic and they are organized in six pathotypes
- Some *E. coli* cause disease by making a Shiga toxin (STEC) and the most predominant enterohemorrhagic (EHEC) strain is O157:H7
- O157:H7 causes bloody diarrhea, dehydration and in most severe cases kidney failure making potentially life threatening
- **Produce contamination source:** production and postharvest water contaminated with feces, contact with infected animals and/or their feces, inadequate worker hygiene
- **Commonly contaminated fresh produce but not limited to:** leafy greens and sprouts

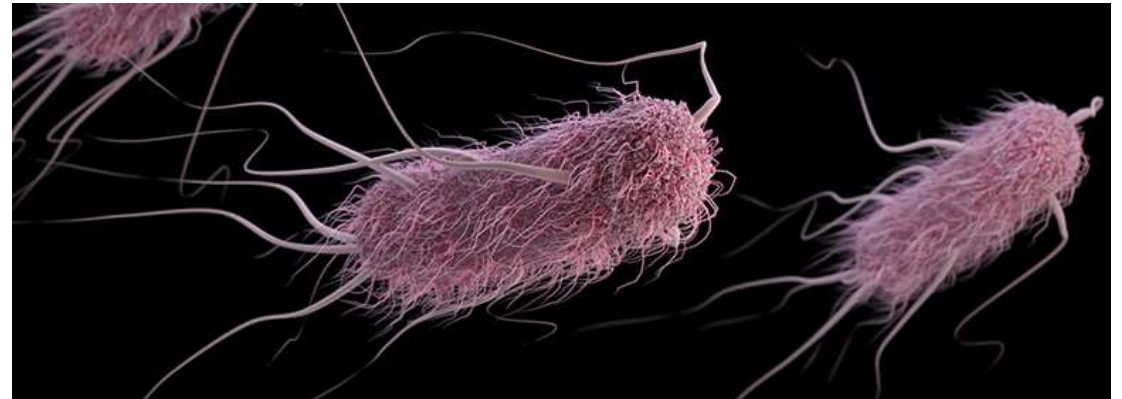


Figure 3. *Escherichia coli* – computer generated image based on scanning electron microscopic photograph.

Listeria monocytogenes

- Motile, Gram-positive, non-sporeforming, biofilm producing rods
- Ubiquitous in the environment (e.g. soil, decaying organic matter)
- Hardy – can withstand and grow in refrigeration temperatures; salt-tolerant
- Causes **listeriosis** which leads in the fatality rates among foodborne illnesses with 20-30% death rate; the symptoms are fever, muscle aches, nausea and/or diarrhea
- Particularly dangerous for immunocompromised population (children under 5 years old, pregnant women, elderly people)
- **Produce contamination source:** soil, production and postharvest water contaminated, contaminated packaging facilities
- **Commonly contaminated fresh produce:** all produce is at risk, including processed and frozen



Figure 4. *Listeria monocytogenes* – computer generated image based on scanning electron microscopic photograph.

Bacillus cereus

- Motile, Gram-positive sporeforming, biofilm producing rod
- Facultatively anaerobic (can survive without oxygen)
- Ubiquitous in the environment (e.g. soil and plants)
- Produces two types of toxin that can cause two types of food poisoning:
 - **diarrheal type** – watery diarrhea, abdominal cramps and pain
 - **emetic type** – nausea and vomiting
- **Produce contamination source:** soil
- **Commonly contaminated fresh produce:** produce contaminated with soil, grain products

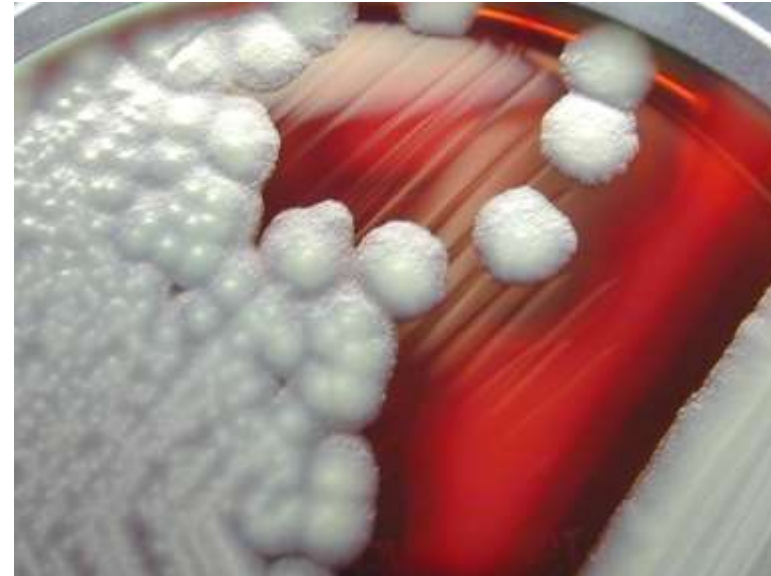


Figure 5. *Bacillus cereus* – culture growth on a sheep blood agar plate.

Clostridium perfringens

- Motile, Gram-positive sporeforming rod
- Anaerobe but aerotolerant (grows without oxygen but tolerates its low levels)
- Ubiquitous in the environment: soil, sediments, also in human and animal intestine
- enterotoxin producing
- Causes two types of food poisoning:
 - **Gastroenteritis** – common, mild and self-limiting
 - **Pig-bel form (enteritis necroticans)** – rare but fatal

- **Produce contamination source:** soil
- **Commonly contaminated fresh produce:** produce contaminated with soil

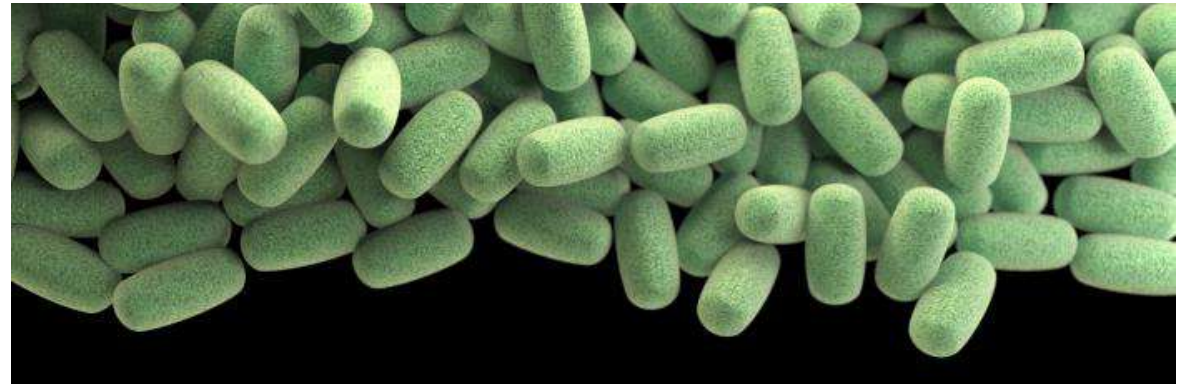
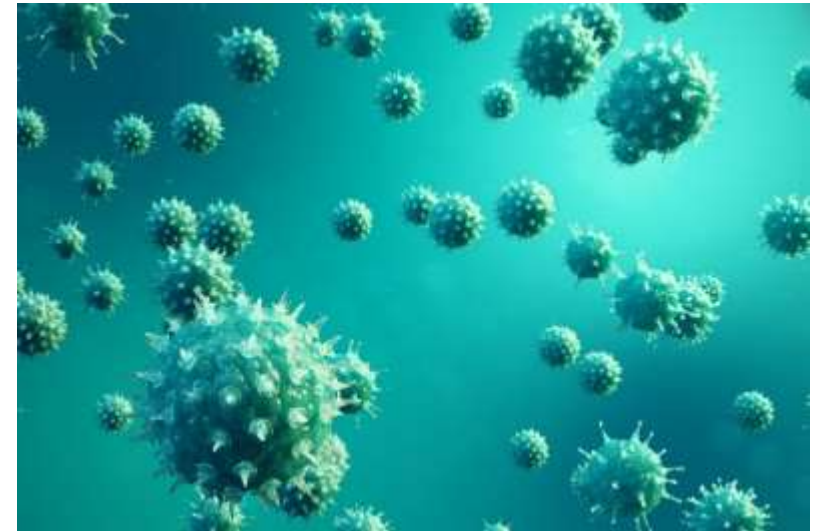


Figure 6. *Clostridium perfringens* – computer generated image based on scanning electron microscopic photograph.

Viruses

- The smallest infectious agents classified by
 - shape,
 - presence of an outside lipid layer (envelope)
 - type of genetic material (DNA, RNA)
- Infect plant, animals, humans and bacteria (phages)
- Ubiquitous in the environment
- Need a living host to reproduce
- General transmission routes:
 - Ingestion of contaminated food and water (fecal-oral route)
 - Respiratory (airborne)
 - Blood and sexual contact
 - Biting arthropods (e.g. mosquitoes or ticks)



Human Norovirus (HuNoV)

- Nonenveloped, single-stranded RNA virus
- The most common etiological agent of foodborne illnesses; > 9 million cases per year in the United States
- Highly contagious and spreads easily in common settings such as healthcare facilities, schools and daycares, restaurants and cruise ship
- Causes **gastroenteritis** with severe vomiting and diarrhea
- Survives freezing
- **Fresh produce:** contaminated water, inadequate worker hygiene, sick workers, contaminated surfaces
- **Commonly contaminated fresh produce:** any fresh produce contaminated with virus, but leafy greens and fruits were found to mostly commonly cause the outbreak

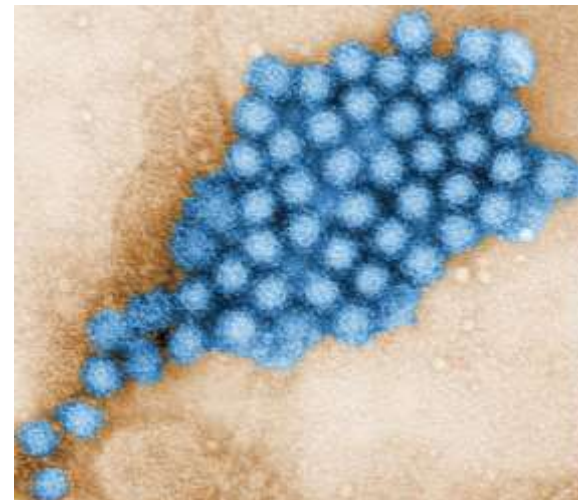


Figure 7: Norovirus virions (digitally colorized); transmission electron microscopy.

Hepatitis A

- Nonenveloped, single-stranded RNA virus
- Causes liver disease; symptoms include diarrhea, nausea, vomiting and jaundice
- Effective vaccination is available
- **Produce contamination source:** contaminated water, contaminated surfaces, sick workers
- **Commonly contaminated fresh produce:** any fresh produce contaminated with virus, but leafy greens and fruits were found to mostly commonly cause outbreaks

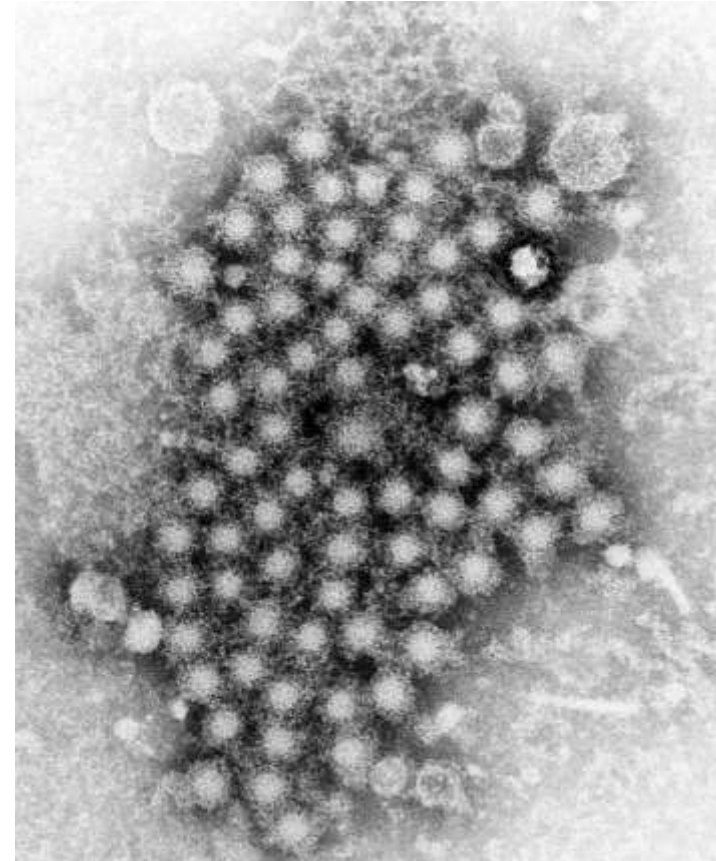


Figure 8. Hepatitis virions of unknown strain; transmission electron microscopy.

Parasites

- Live on or in a host, and at its expense
- Only reproduce in a host but can survive in the environment
- Can have very complicated life cycle
- There are three main classes:
 - Protozoa – microscopic, single-cell organisms that can be free-living, e.g. *Giardia*, *Cyclospora*, *Toxoplasma gondii*
 - Helminths – multicellular organisms, visible to a naked eye, e.g. flat- and roundworms
 - Ectoparasites – blood sucking arthropods

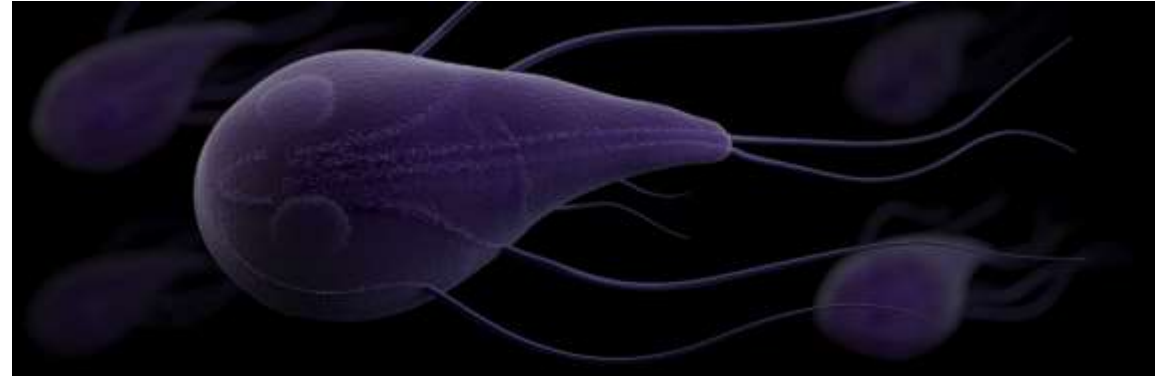


Figure 9. *Giardia lamblia* – computer generated image based on scanning electron microscopic photograph.

Toxoplasma gondii

- A single-cell protozoan cell
- Very ubiquitous in the environment; as many as 95% of some populations may be infected
- Infection by ingestion of sporulated oocysts with food or water
- Contamination through cat feces with oocysts
- Causes **toxoplasmosis**, a leading foodborne disease that can result in death
- **Produce contamination source:** soil, water, and surfaces contaminated with oocysts from cat feces
- **Commonly contaminated fresh produce:** any fresh produce contaminated with sporulated oocysts



Figure 10. *Toxoplasma gondii* - oocysts in a fecal floatation.

Giardia

- A single-cell protozoan, protected by outer shell (resistance to environmental conditions or chlorine treatment)
- Motile
- Resides in soil, food or water contaminated with human or animal feces
- Infection by ingestion of cysts in contaminated food or water
- Causes **giardiasis**, mostly self-limiting and asymptomatic; present syndromes include diarrhea, malaise, cramps, flatulence, and weight loss
- **Produce contamination source:** contaminated production and/or postharvest water, sick workers
- **Commonly contaminated fresh produce:** any fresh produce contaminated with the parasite, but leafy greens and berries are mostly susceptible to contamination due to their complex surface structure

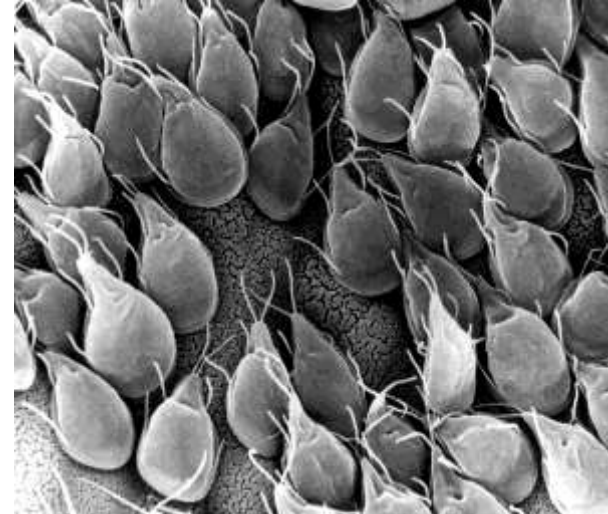


Figure 11. *Giardia* sp. trophozoites; scanning electron microscopy.

Cyclospora

- Single-cell protozoan parasite
- Infects epithelial cells of the intestinal tract
- Contamination through feces with oocysts
- Infection by ingestion of sporulated oocysts with contaminated water or food
- **Produce contamination source:** contaminated agricultural water, contaminated surfaces, ill workers
- **Commonly contaminated fresh produce:** any fresh produce contaminated with the parasite but most outbreaks were associated with imported raspberries and basil

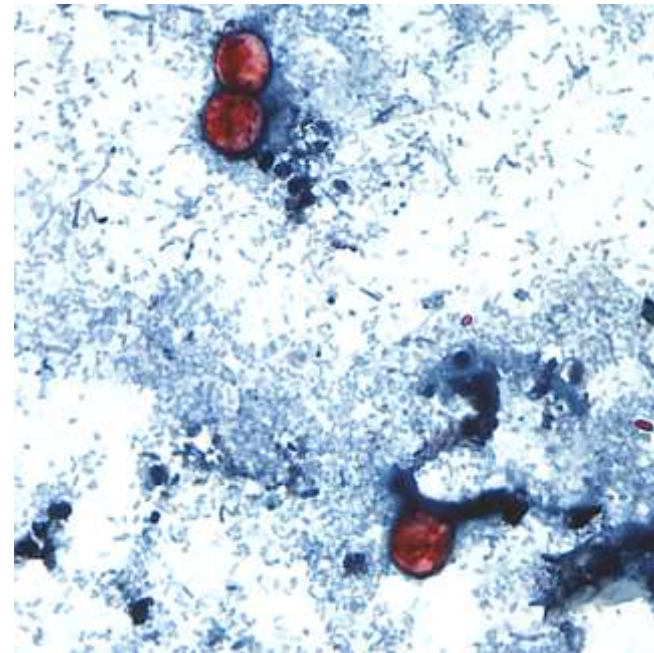
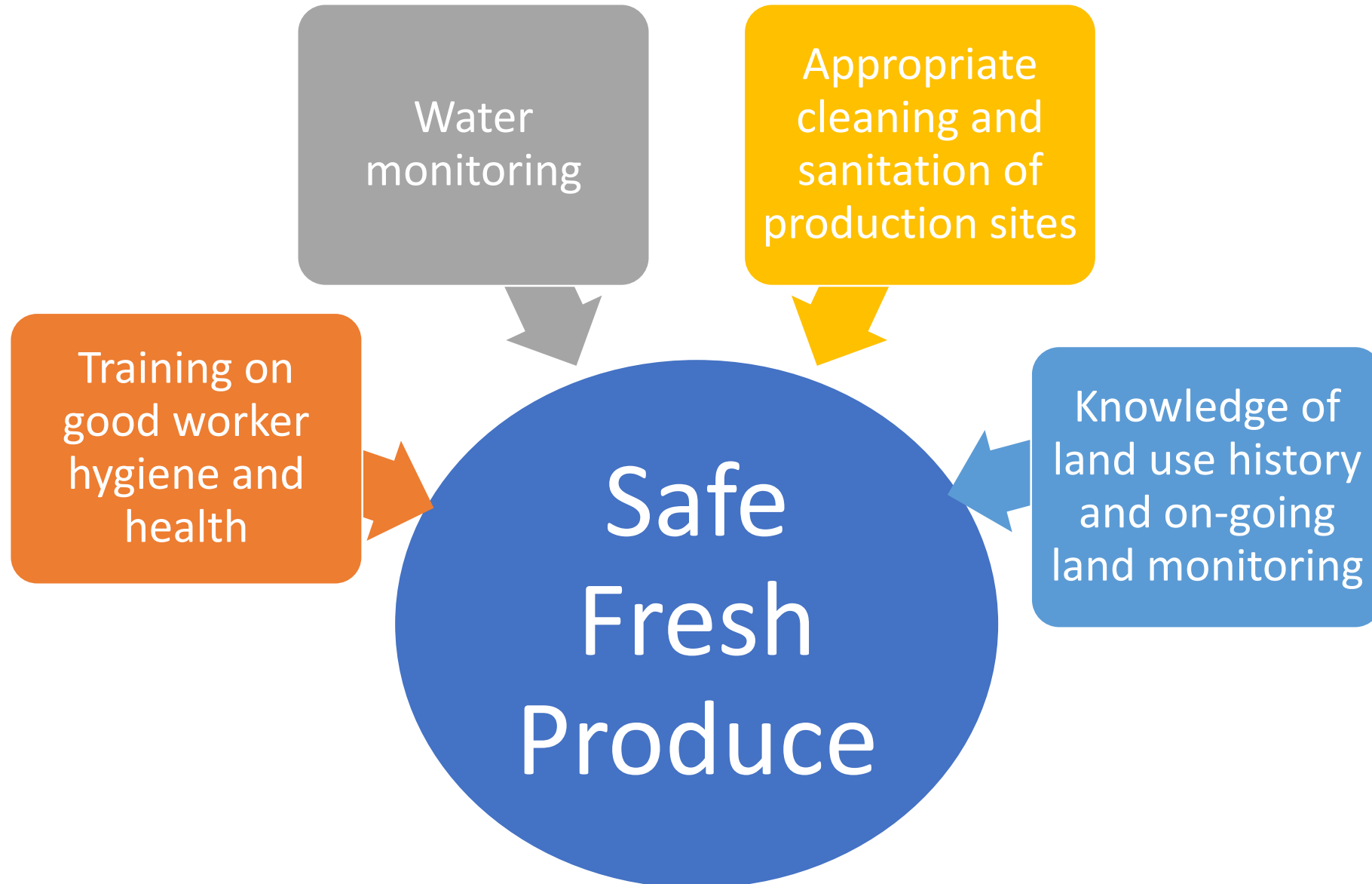


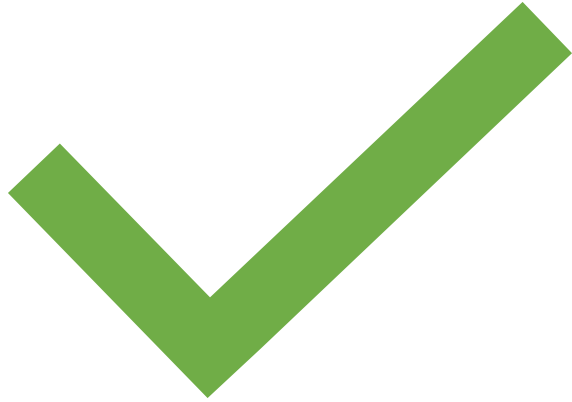
Figure 12. *Cyclospora cayetanensis* oocysts stained with safranin.



Steps to Prevent Contamination of Produce

Prevention – Critical Points





Worker Health and Hygiene

- Training and monitoring of worker hygiene and harvest practices
- Reporting sickness and injuries
- Availability and easy access to potable water and sanitary stations in the field



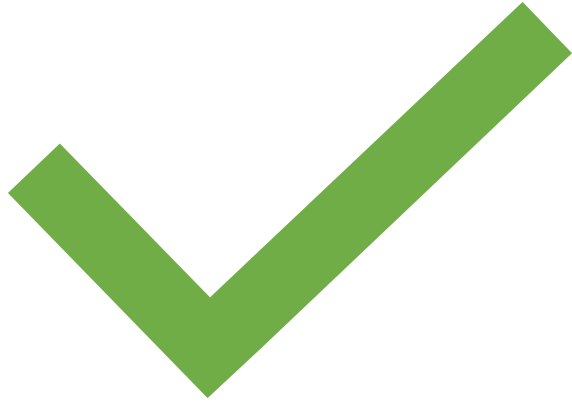
Soil and Biological Soil Amendments

- Soil testing for the previous land use if there is an evidence of contamination event such as flooding;
- Using treated biological soil amendments
- Good timing for manure application – at least two weeks before planting, 120 days before the harvest for produce with edible parts in contact with soil, 90 days before the harvest for produce with edible parts not in contact with soil;
- Following the scientifically validated methods for composting



Wildlife and Domestic Animals

- Determent of wildlife to minimize the animal presence – decoys, fences, nets, noise deterrents, tactile repellents, relocation
- Excluding and controlling the domesticated animals from entering the produce fields
- Banning pets from entering produce fields



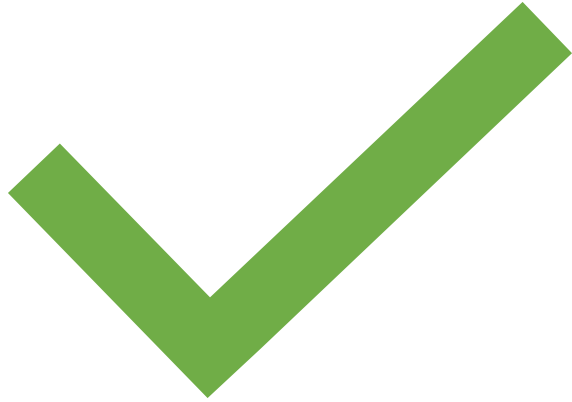
Agricultural Water – Production

- Determination of risk assessment of adjacent land use and any events that could result in the contamination of the water source
- Monitoring and controlling animal access to the production water



Agricultural Water - Postharvest

- Safe water source; postharvest water testing must show no detectable *Escherichia coli* in 100 mL of tested water
- Use of antimicrobial products in water (diluted bleach, peracetic acid, hydrogen peroxide)
- Implementation of correct cleaning and sanitation program



Postharvest Handling and Sanitation

- Good worker hygiene (washing hands, wearing clean clothing, gloves etc.)
- Good worker health (reporting sickness and injuries);
- Implementation of cleaning and sanitation programs
- Pest management



For More Information

- Produce Safety Alliance Extension
- Your State's Department of Agriculture
- FDA
- Good Agricultural Practices (GAPs)
- USDA



Small Farm & Ranch Webinar Series

Cultivating Success offers a **FREE** lunchtime webinar series designed to help you explore, strengthen or expand your small farm or ranch operation!

Mondays and Tuesdays at 11 am PST / 12 pm MST



Tuesday, March 10th – Financial Fitness for Farmers Part 2: Income Statements

Monday, March 16th – Assessing Beneficial Insect Habitat on Your Farm

Tuesday, March 17th – Financial Fitness for Farmers Part 3: Enterprise Budgets

Tuesday, March 24th – Financial Fitness for Farmers Part 2: Cash Flow Statements

<https://www.cultivatingsuccess.org/webinar-series>



Please take our post-webinar survey!

Our post-webinar surveys are short and quick! And, your feedback is important!

Click on the link below to take the survey:

https://www.surveymonkey.com/r/Pathogenic_Microbiology



University of Idaho
Extension



Food Systems

WASHINGTON STATE UNIVERSITY



To learn about upcoming programs and watch recorded webinars, please visit

www.cultivatinguccess.org