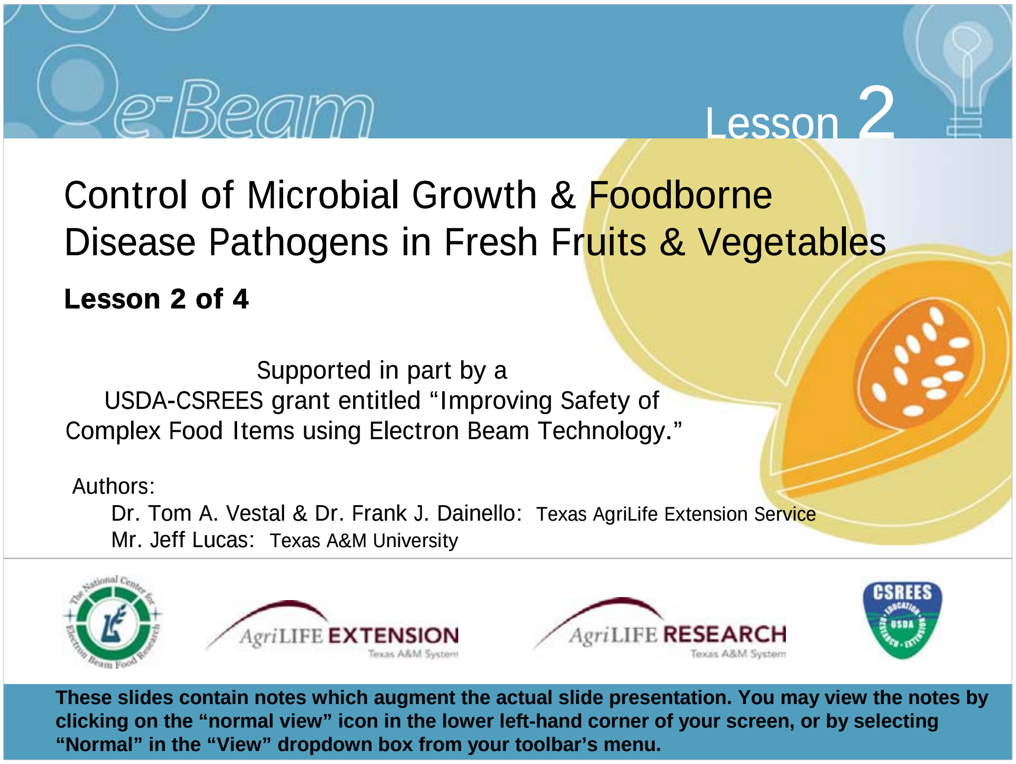
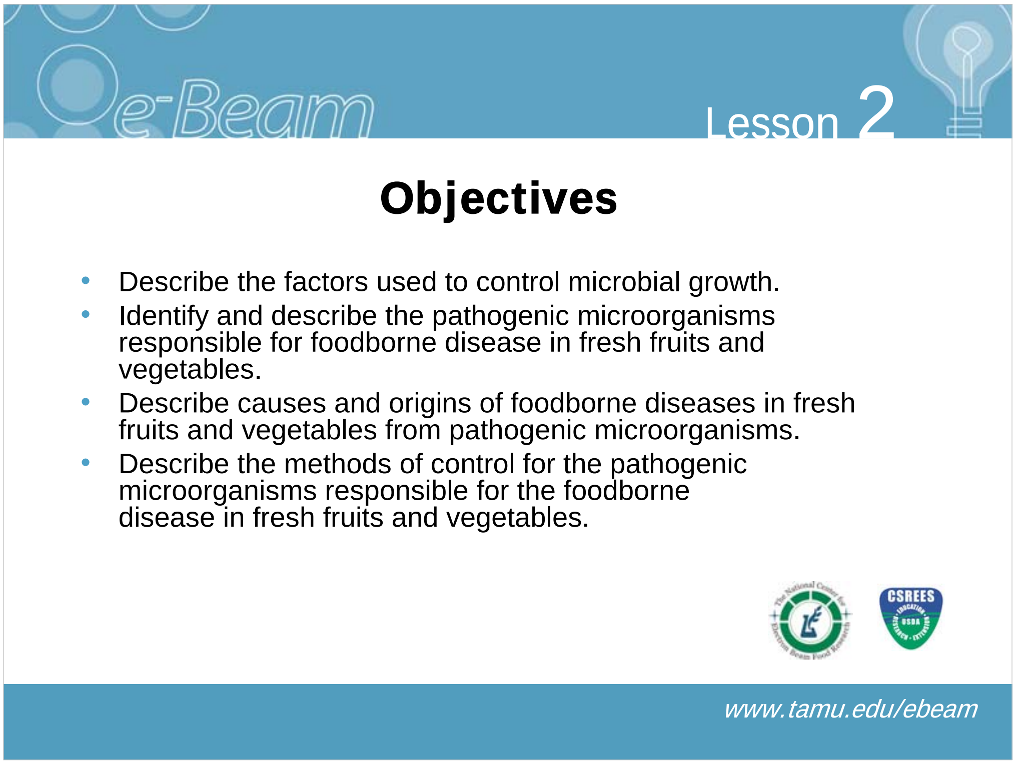


Lesson two, Control of Microbial Growth and Foodborne Disease Pathogens in Fresh Fruits and Vegetables is brought to you from ,Texas AgriLife Extension Service and the National Center for Electron Beam Food Research at Texas A&M University. A grant proposal entitled “Improving Safety of Complex Food Items using Electron Beam Technology” was awarded to Texas Agricultural Experiment Station and Texas AgriLife Extension Service by USDA-CSREES to support this lesson. Texas AgriLife Extension Service food safety experts seek this opportunity to share knowledge with you so that the food that you produce, process, prepare, and consume are of high quality, wholesome, and safe from pathogens.



Learners who complete this lesson will be able to…

…describe the factors used to control microbial growth.

…identify and describe the pathogenic microorganisms responsible for foodborne disease in fresh fruits and vegetables.

…describe the disease etiology (the study of causes and origins) for the pathogenic microorganisms responsible for foodborne disease in fresh fruits and vegetables.

…describe the methods of control for the pathogenic microorganisms responsible for the foodborne disease in fresh fruits and vegetables.

Microorganisms which cause foodborne disease from consumption of fresh fruits and vegetables can be grouped into 3 majors categories: bacteria, viruses and parasites.

These three categories have major differences in how they are constructed. The viruses, in particular, are distinctly different in that they use the host to manufacture other viral particles. Bacteria and parasites use their host as a source of nutrients for growth and multiplication.

Even though these organisms have distinct differences, the cause of their prominence regarding foodborne disease has major similarities; these similarities include but are not limited to:

1. Improper personal hygiene practices by food handlers,
2. Use of animal wastes as fertilizer, and
3. Use of contaminated wash-water and irrigation water.
4. Cross contamination from meat, poultry, or fish.

Fresh-cut produce, particularly some fruits, have natural compounds (acids) that inhibit the growth of microorganisms. These compounds are inhibitors to microbial growth that we can use to our advantage in food processing.

Naturally occurring compounds or product characteristics which inhibit growth of microorganisms are called intrinsic factors.

Storage conditions or characteristics which can be manipulated to restrict or inhibit microbial growth are called extrinsic factors.

e-Beam Lesson 2

Bacterial Agents of Foodborne Disease

- **Gram negative***
  - *Salmonella*
  - *Shigella spp.*
  - Pathogenic *Escherichia coli*
  - *Escherichia coli O157:H7*
  - *Campylobacter*
- **Gram positive***
  - *Listeria monocytogenes*
  - *Clostridium botulinum*
  - *Bacillus cereus*

* Defined in the notes section.

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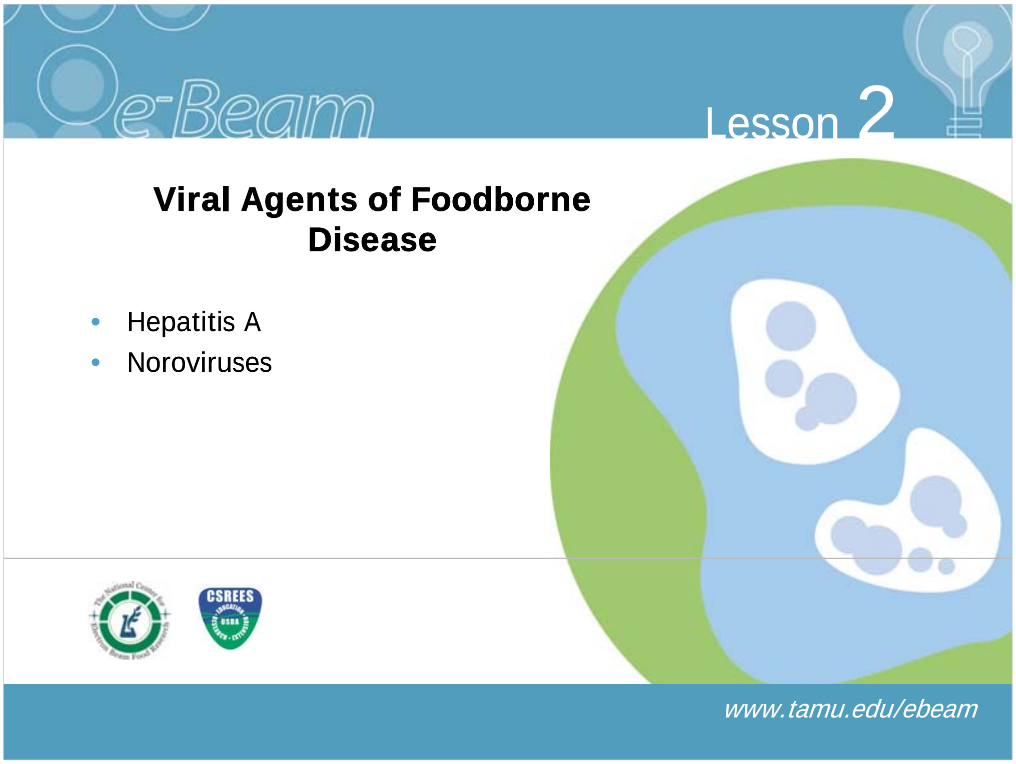
Microorganisms vary in their ability to respond favorably to intrinsic and extrinsic factors of microbial inhibition. To ensure that target organisms are controlled, we must review organisms of concern and their individual growth parameters.

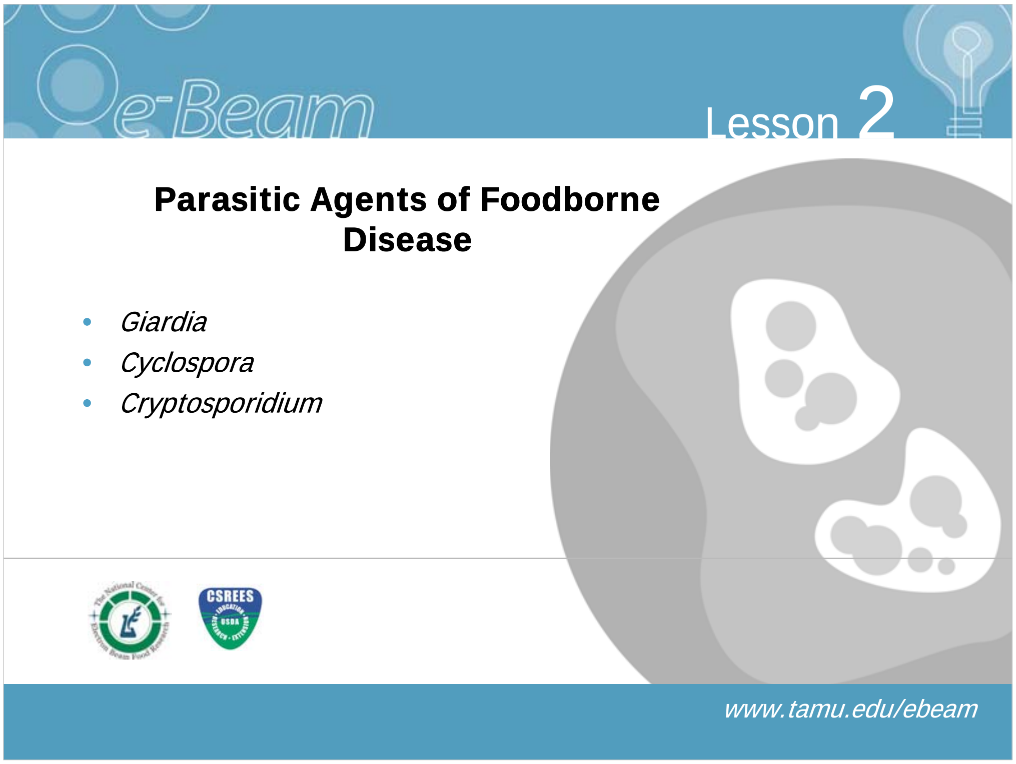
By far, our greatest concern with foodborne disease agents involves those agents which are bacterial in origin. Microbiologists divide bacterial organisms into two major classes: Gram positive and Gram negative. The difference between these classes involve the cell wall. Simply put, the Gram positive cell wall has components that trap the primary stain, crystal violet, and this stain is not washed away with alcohol. If the cell is a Gram negative cell, the purple crystal violet is washed away and the cell will turn pink when a second dye of safranin is applied. Therefore, Gram positive cells are purple and Gram negative cells are pink.

A detailed discussion of each bacterial pathogen of concern in fresh-cut produce will follow later in this module.



Viruses are dramatically different than either bacteria or parasites. They are considered non-cellular entities which consist of proteins and nucleic acid. Viruses replicate (or multiply) only after entering a host cell. Viruses contain no intrinsic metabolic processes, and directs the host cell to generate more viral particles.

Two viruses of concern in fresh-cut produce are Hepatitis A and noroviruses.



Parasites are different than either viruses or bacteria. A parasite may involve both a primary and secondary host to complete its life cycle or may simply involve one host.

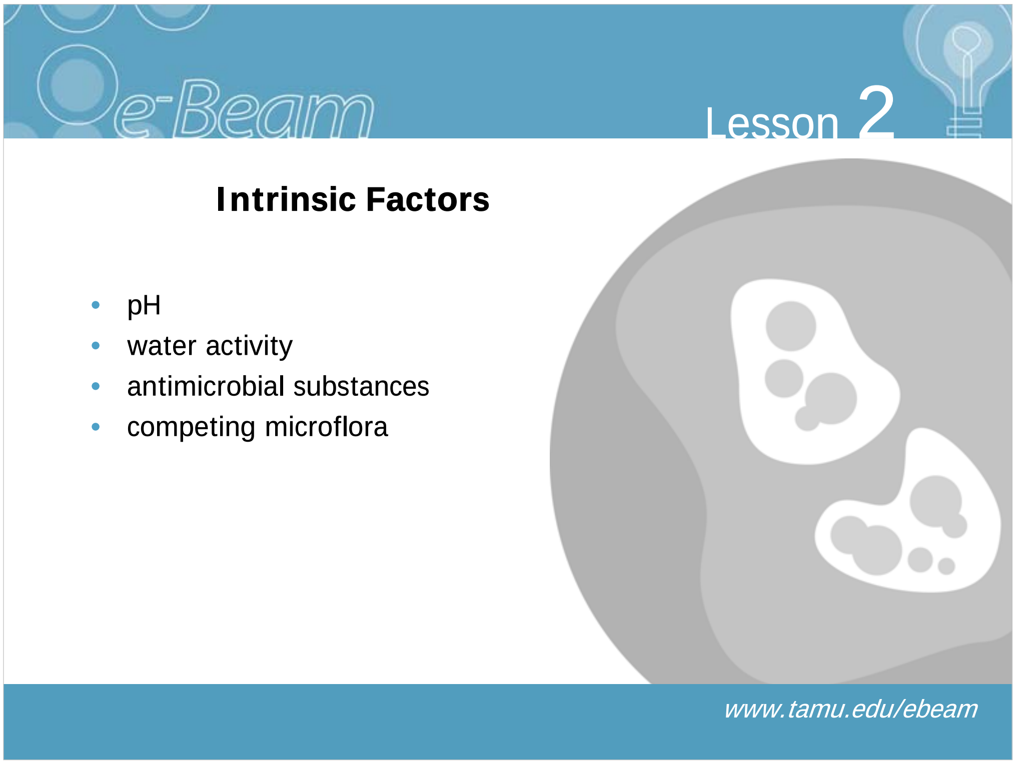
Foodborne diseases resulting from parasites involve consumption of the cyst form of the organism through contaminated water used on the produce (fruits and vegetables) or through produce contaminated by the food handler. The parasites of concern are:

Giardia

Cyclospora

Cryptosporidium

Before we go into detail regarding the specifics of the individual organisms, lets take a look at intrinsic and extrinsic factors in more detail.

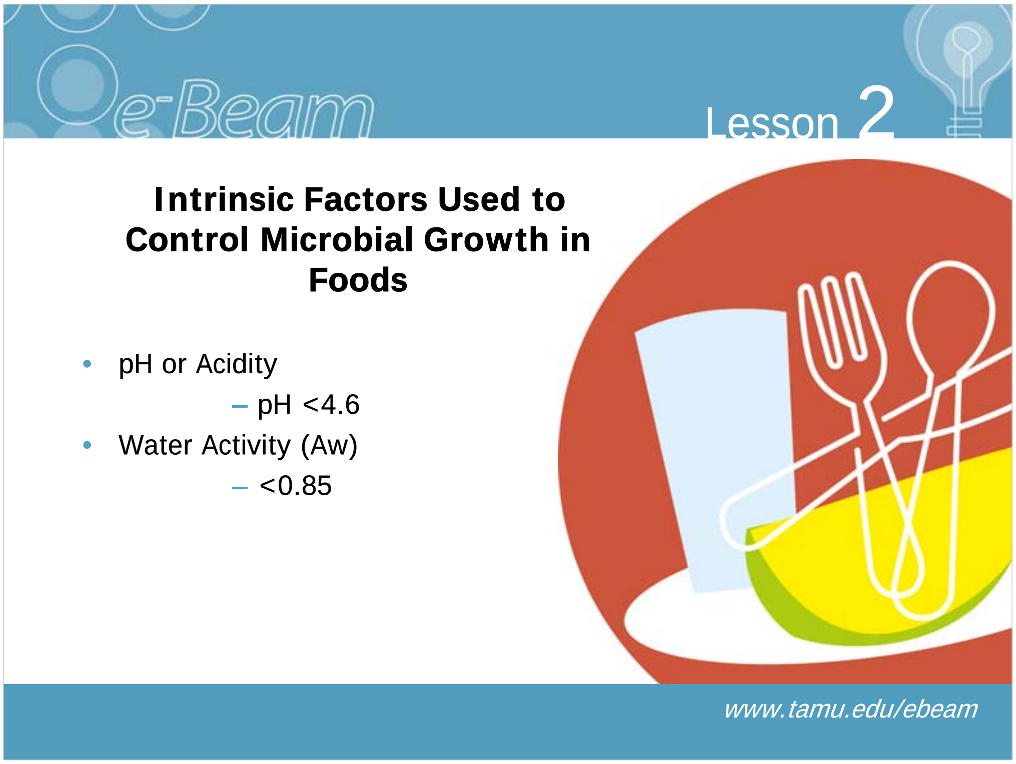


Fruits and vegetables are an excellent source of nutrients for the growth of microorganisms. Fresh-cut produce contains an abundant source of water, starch, and certain vitamins and minerals.

In general, vegetables have a pH range between 5 to 7. Microorganisms grow well in this pH range. Fruits, on the other hand, have a lower pH. A pH of less than 4.6, generally speaking, is inhibitory to the growth of microorganisms. For example apples have a pH of 2.9 to 3.6, although microorganisms may survive in this pH range they will not multiply. This doesn't mean that apples or apple juice is completely safe. It means that if the level of the microorganism is below the infective dose (that level shown to induce foodborne disease), then the pH will stabilize the product and prevent further multiplication of the pathogen. Antimicrobial substances, such as organic acids, can be added to reduce pH levels and inhibit pathogen growth.

Drying fruits and vegetables has been used for centuries to preserve foods. Dates, apples, peaches, and prunes are routinely dried to prevent microbial growth. The water activity, or relative humidity, of the product must be below 0.85 for the product to be considered shelf-stable. The low water activity restricts pathogen growth because water isn't available for growth.

The phenomenon of lactic antagonism takes place when certain spoilage bacteria (lactic acid bacteria) produce bacteriocins, or growth inhibiting compounds restrict the growth of other bacteria.



Many factors can be used to control microbial growth. pH (or acidity) of a food or agricultural commodity is one of the most effective means of preventing microbial growth. During growth, microorganisms must rid themselves of acid by-products. A high acid content in food or agricultural products prevents the microbial cell from eliminating waste acid within the cell. This build-up of acid within the cell eventually stops microbial cellular growth and multiplication.

Because fruits bind water with sugar, fruits often have low available water for cellular growth. This low water activity simply restricts cellular growth due to a lack of moisture. Water activity is used to measure a food's moisture content with respect to microbial growth and ranges from 0.00 to 1.00 (pure water).

Some foods may be preserved (spoilage microbes destroyed) by removing their water content. For example the minimum water activity needed for *Escherichia coli* (*E. Coli*) to survive is 0.96, while spoilage molds may survive with a much lower water activity of 0.80.

Lactic acid bacteria produces the organic acids which restrict the growth of other microorganisms. This approach has been used for centuries in fermentation of wine, beer, certain fruit products, sourdough, and in fermented sausages for product preservation.

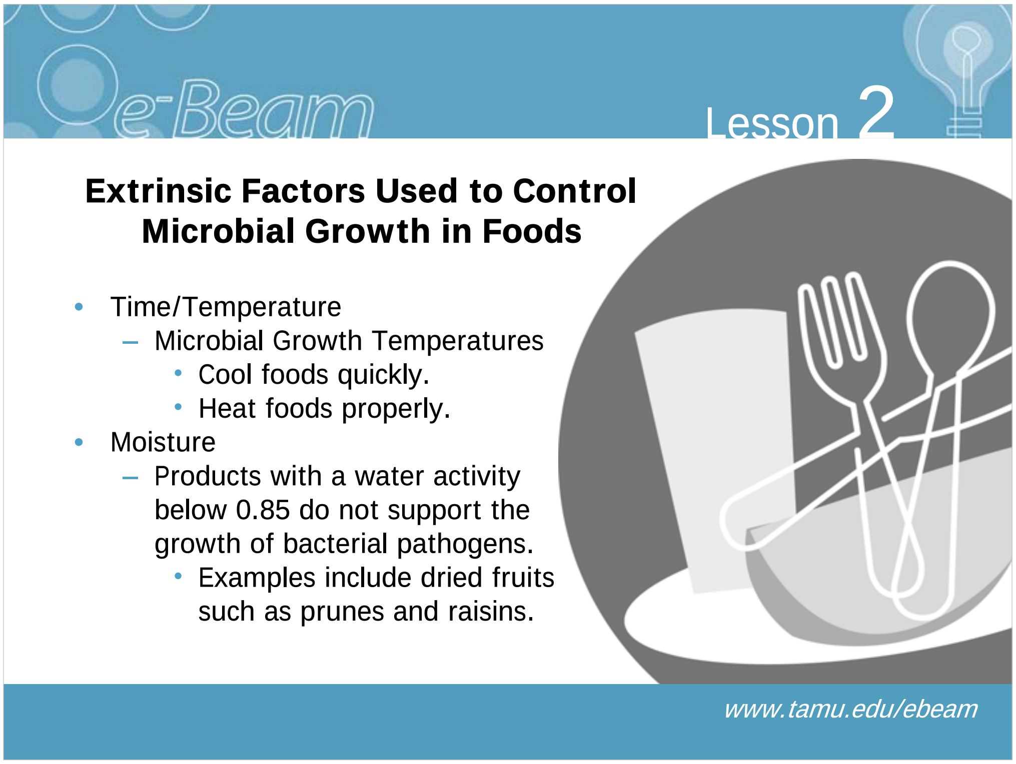
Acids can be generated naturally through fermentation or added as an ingredient to inhibit microbial growth.

Heating has been used for centuries to make foods safe for consumption. Since the advent of refrigeration, chilling or cooling of foods has been used for food preservation. However, many fruits and vegetables are consumed raw. Therefore, other means must be used to ensure the safety of products we eat.

Drying removes the moisture such that water activity is decreased to a level in which pathogens cannot grow. We must recognize that while drying prevents growth, certain pathogens may survive the drying process.

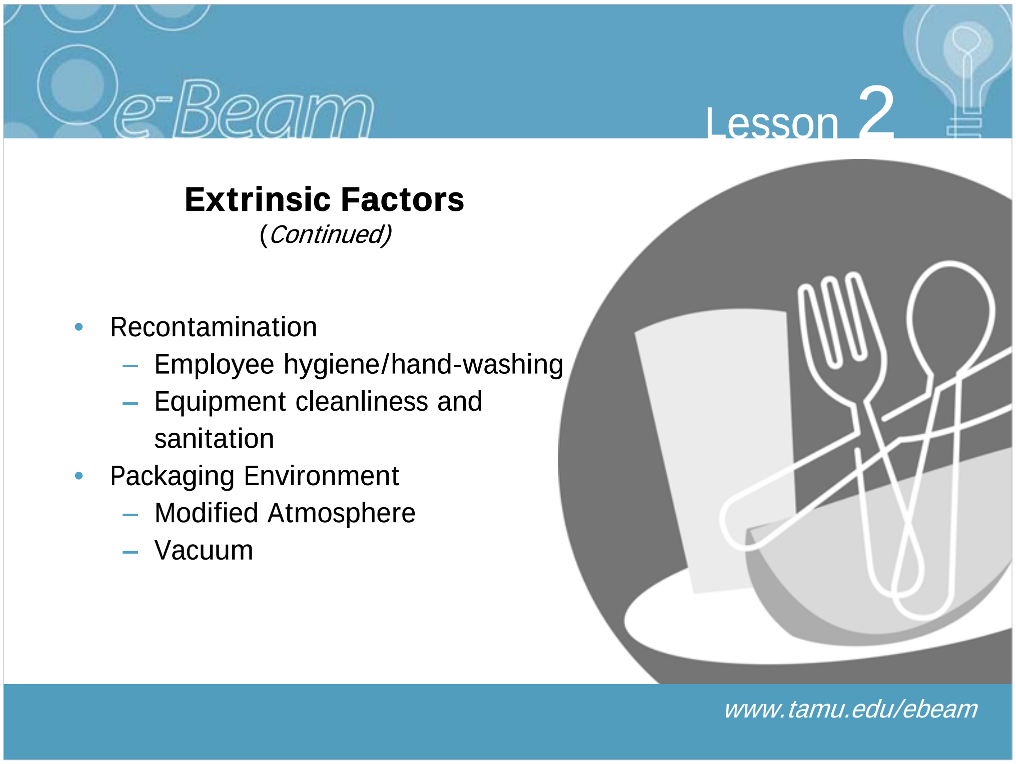
In the last 20 years, the most important concept in producing a safe processed product is the prevention of recontamination after processing. The emergence of *Listeria monocytogenes* which grows slowly at refrigeration temperatures will be discussed in detail later in this lesson, has heightened the degree of awareness regarding the importance of food processing plant sanitation.

Packaging systems used have a substantial effect on the microflora that exists throughout shelf-life. Packaging systems which restrict or remove oxygen favor the development of certain bacteria within the package. Certain pathogens will proliferate if oxygen is removed from the package.



Most fresh-cut produce is stored at refrigeration temperatures. Freezing is the only means of stopping microbial growth, but destroys the tissues in most fruits and vegetables so that water loss occurs. With this water loss, there is a loss of both nutrients and the integrity (or mouth feel) of the product.

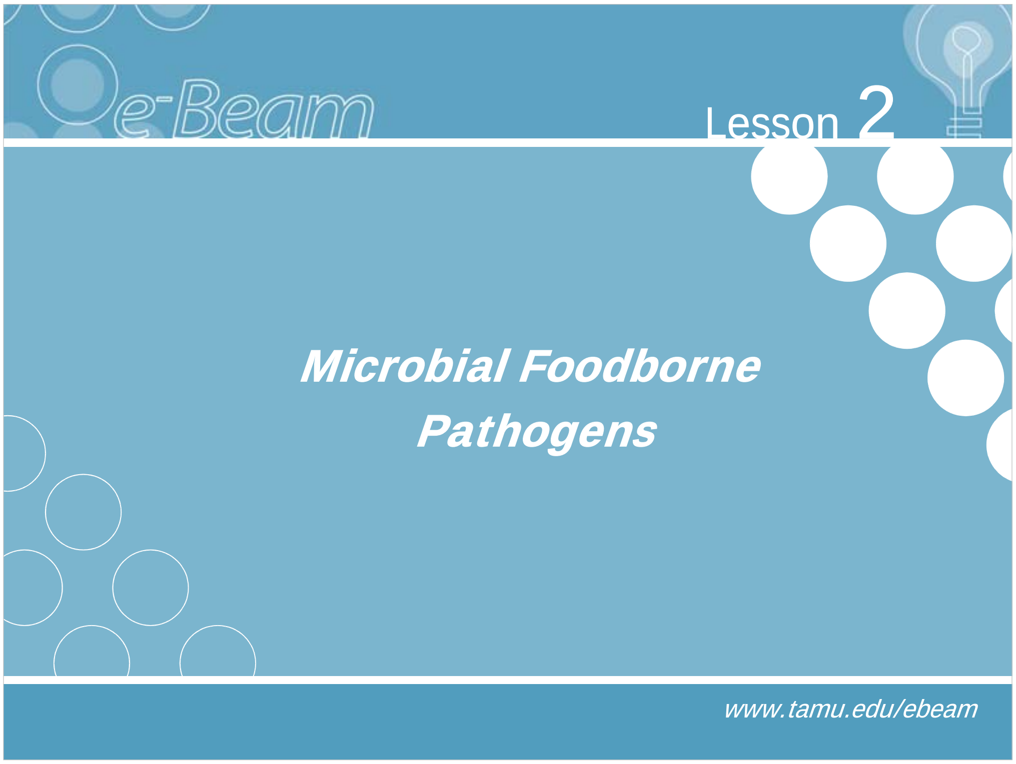
Heating is the only means to completely destroy all microbial pathogens. Because many vegetables and most fruits are consumed raw, heating is not really an option to preserve these products.



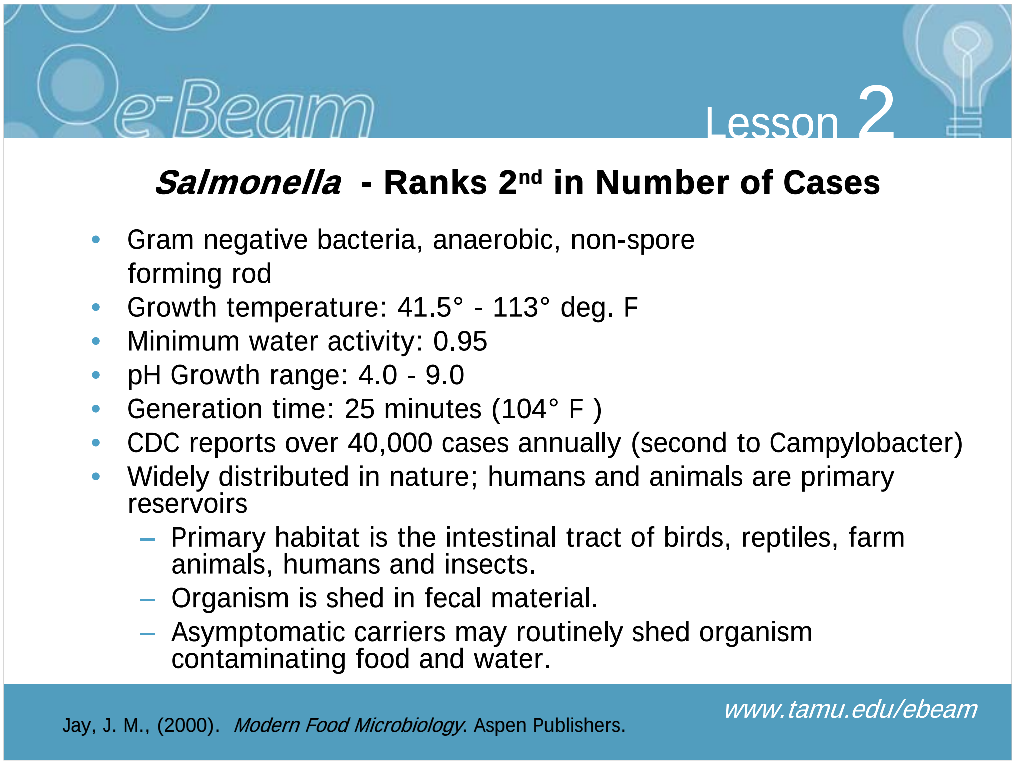
The personal hygiene of food handlers and the sanitation of food processing equipment and utensils seems simple, but are often the root Cause of foodborne disease outbreaks caused in manufacturing and at the home. Engineered food packaging processes, especially modified atmosphere and vacuum packaging, are proven methods and contribute to present day safety and food quality.

In the hurdle concept, we employ multiple intrinsic and extrinsic factors to control microbial growth. For example, in making jellies and fruit preserves, we start with a high acid fruit, cook out the moisture while adding solutes (sugar), then fill the jars while the product is hot.

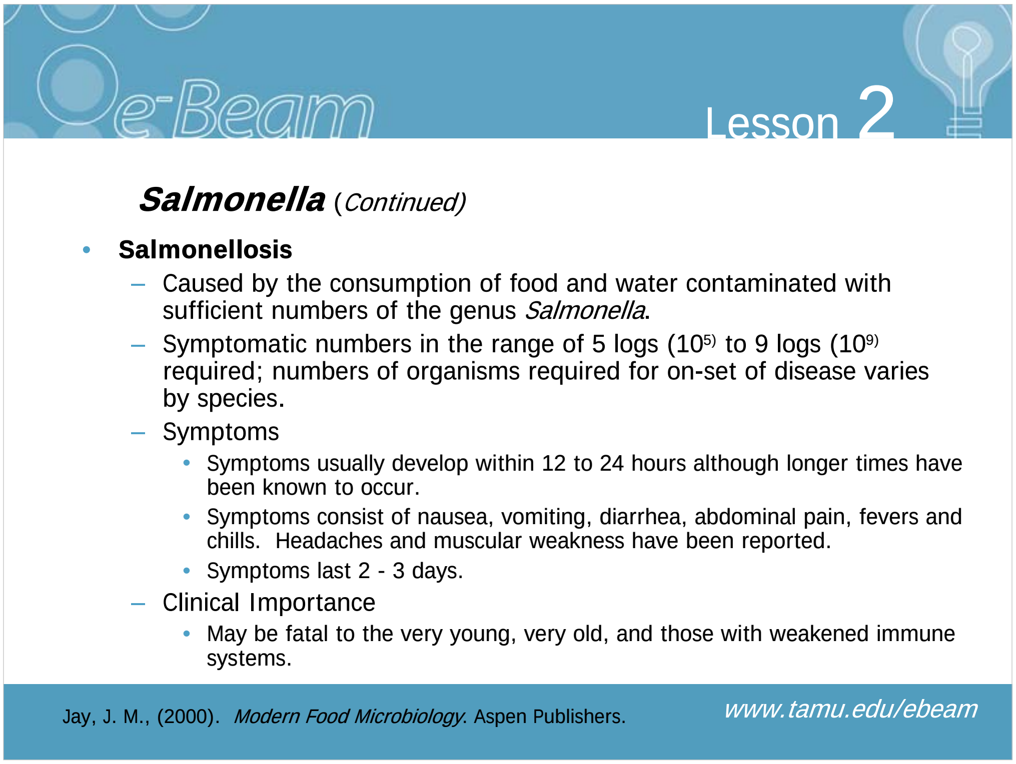
The heat kills the vegetative cells, the acid and low water activity prevents spore-former out-growth and the hot filled product creates a vacuum (lack of oxygen) as it cools. This lack of oxygen prevents microbial spoilage until the jars are opened.



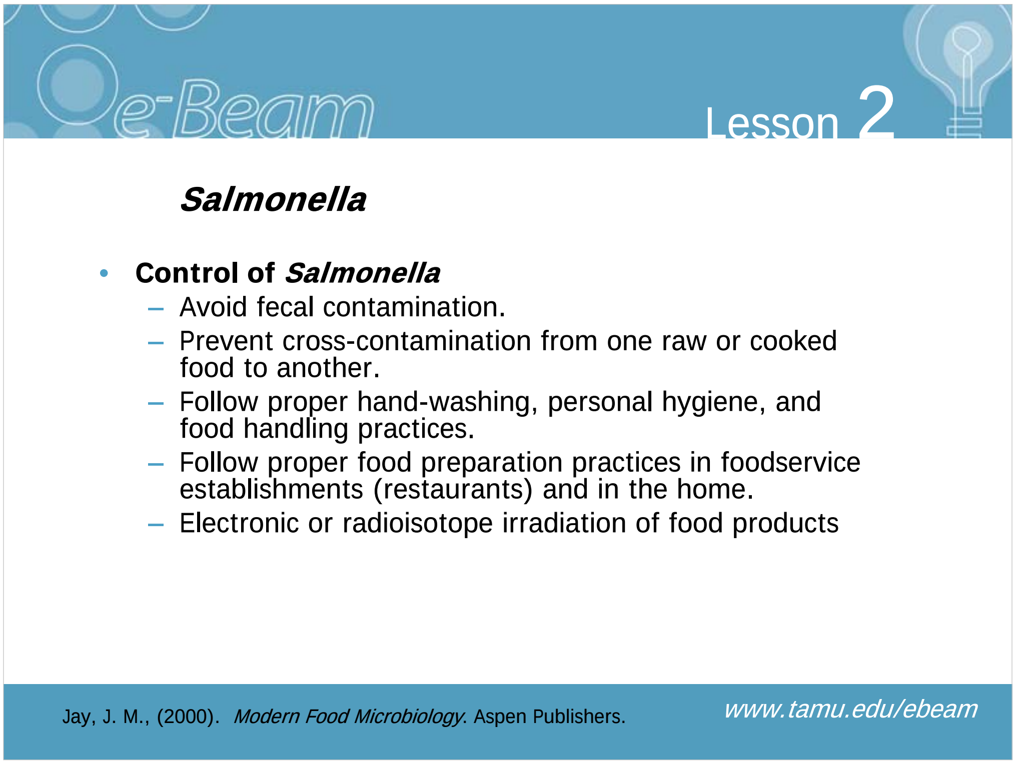
Now lets take a look at specific pathogens.



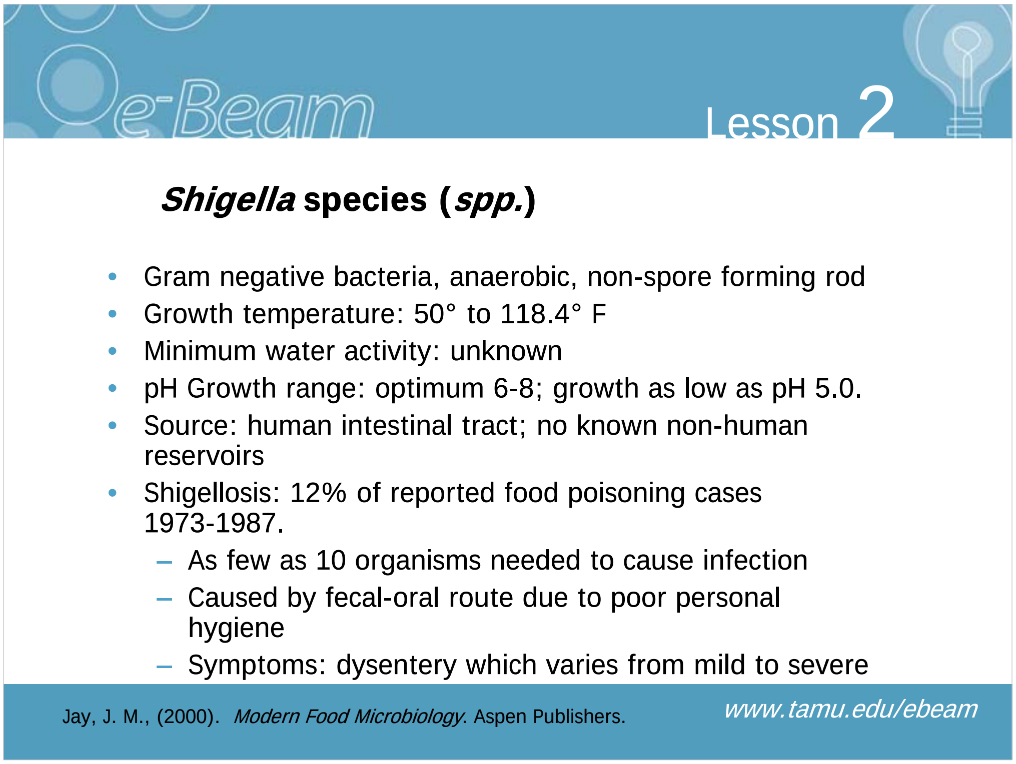
With over 40,000 cases per year, Salmonella ranks 2nd in the number of foodborne disease cases.



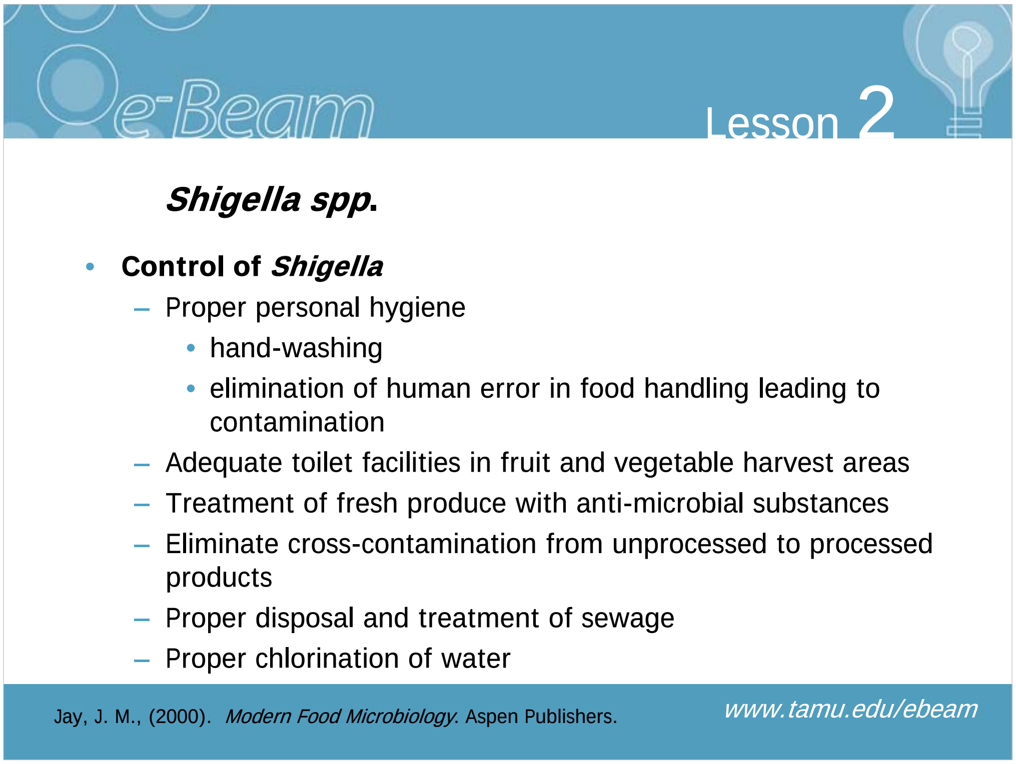
Salmonella rapidly builds in population of 100,000 (10^5) to 1,000,000,000 (10^9) to cause Salmonellosis.



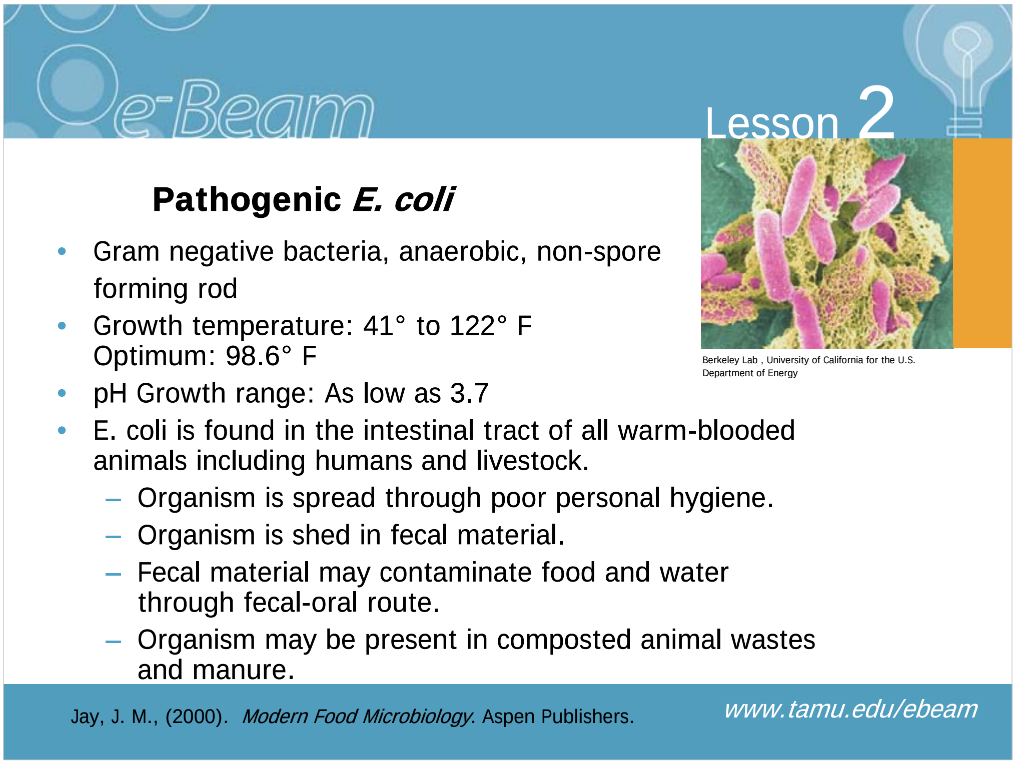
In 2001, the Food and Drug Administration undertook an exhaustive review of the pathogens involved in foodborne disease outbreaks associated with fresh-cut produce. *Salmonella* was implicated in outbreaks associated with cantaloupe, oranges, tangerines, strawberries, bean sprouts, lettuce, salad greens, salad mix, chili peppers, parsley, cilantro, cauliflower, cabbage, celery, eggplant, green onions, and zucchini.



Shigella is a bacterial pathogen which we hear very little about. According to the Institute of Food Technologists, this disease affects the urban poor, immigrants, Native Americans, daycare centers and institutions at disproportionate rates. *Shigella* can have very mild symptoms or very severe symptoms and may have mortality rates approaching 10 to 15 percent. The fact that some portions of the population are particularly at risk, such as the very young in daycare centers, is alarming because this disease may produce hemolytic uremic syndrome (or kidney failure) in the very young. The potency of *Shigella* is due to its ability to produce enterotoxins known as *Shiga* toxins, which are the destructive element of the disease.



"An estimated 20% of the total number of cases of *Shigellosis* involve food as the vehicle of transmission" according to the Institute of Food Technologists. Salads and seafoods handled by infected workers have been implicated in these outbreaks. This organism is readily killed by heat, however many fruits and vegetables are consumed without heat treatment putting consumers at risk. We have reason for concern because many fruits and vegetables are harvested by migrant workers (a population with disproportionate levels of *Shigella* infection) and many harvesting locations lack adequate personal hygiene facilities, we have a reason for concern. Additionally, food processors do not routinely monitor their workers for asymptomatic carriers, nor do they monitor the processing facility environment for the presence of *Shigella*.



Pathogenic *E. coli* is found in the intestinal tract of all warm-blooded animals including humans and livestock and severely dehydrated infants.

e-Beam Lesson 2

Pathogenic *E. coli*

- **Travelers' Diarrhea**
  - Typical of symptoms from *E. coli* infection.
  - Acute watery diarrhea
  - Caused by consumption of contaminated food and/or water
  - Enteropathogenic *E.coli*
    - do not produce enterotoxins
    - generally infects children under 1 year of age
  - Enterotoxigenic *E. coli*
    - produces 2 types of heat stable enterotoxin after colonization of intestine
    - infects children and adults

Jay, J. M., (2000). *Modern Food Microbiology*. Aspen Publishers.

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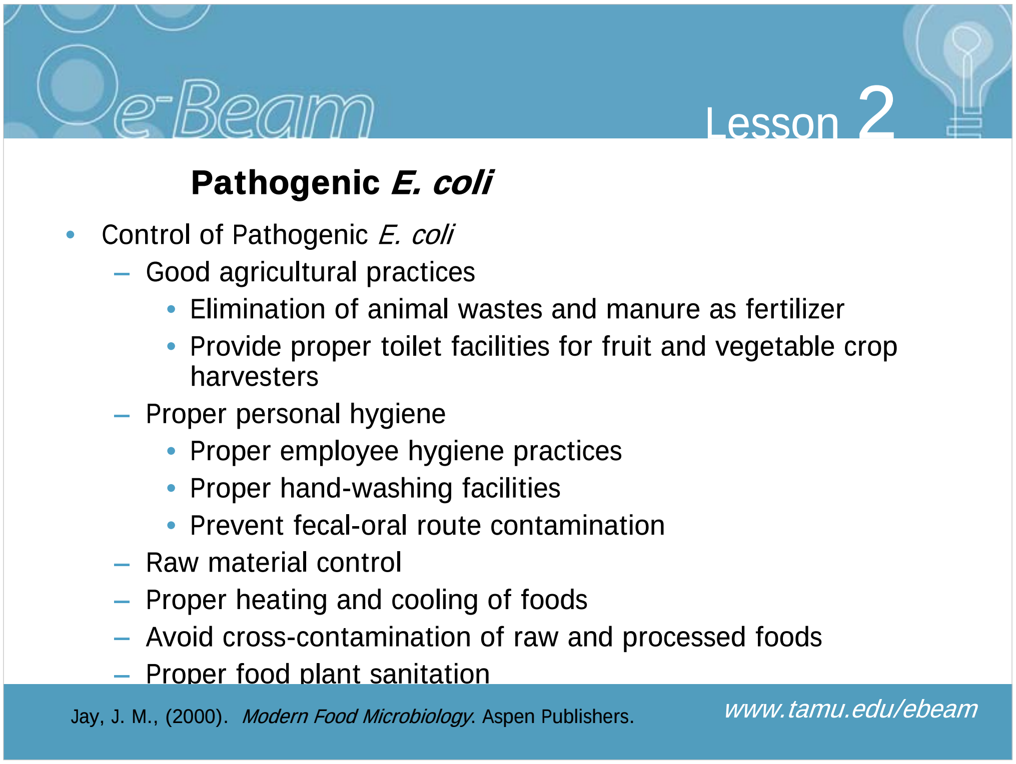
Some refer to the symptoms of Pathogenic *E. coli* as "Travelers' Diarrhea".

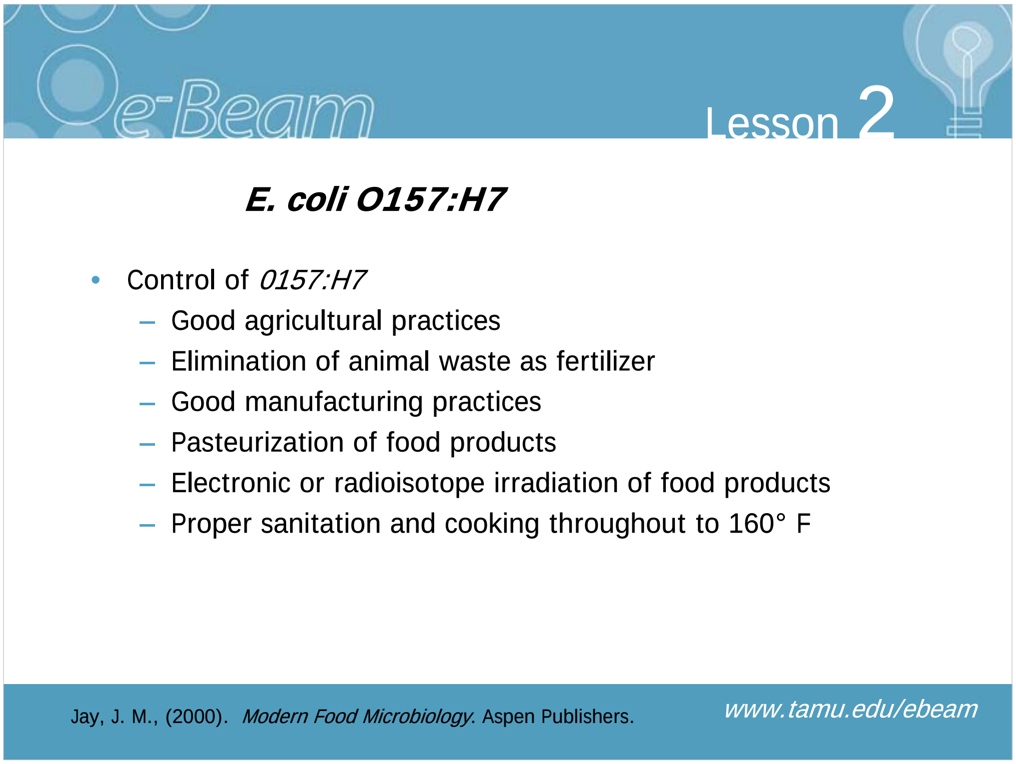
Enterotoxins are toxins produced by bacteria specific to intestinal cells which cause vomiting and diarrhea.



Pathogenic *E. coli* is controlled through good agricultural and food processing practices, proper personal hygiene, and proper food handling and preparation practices.

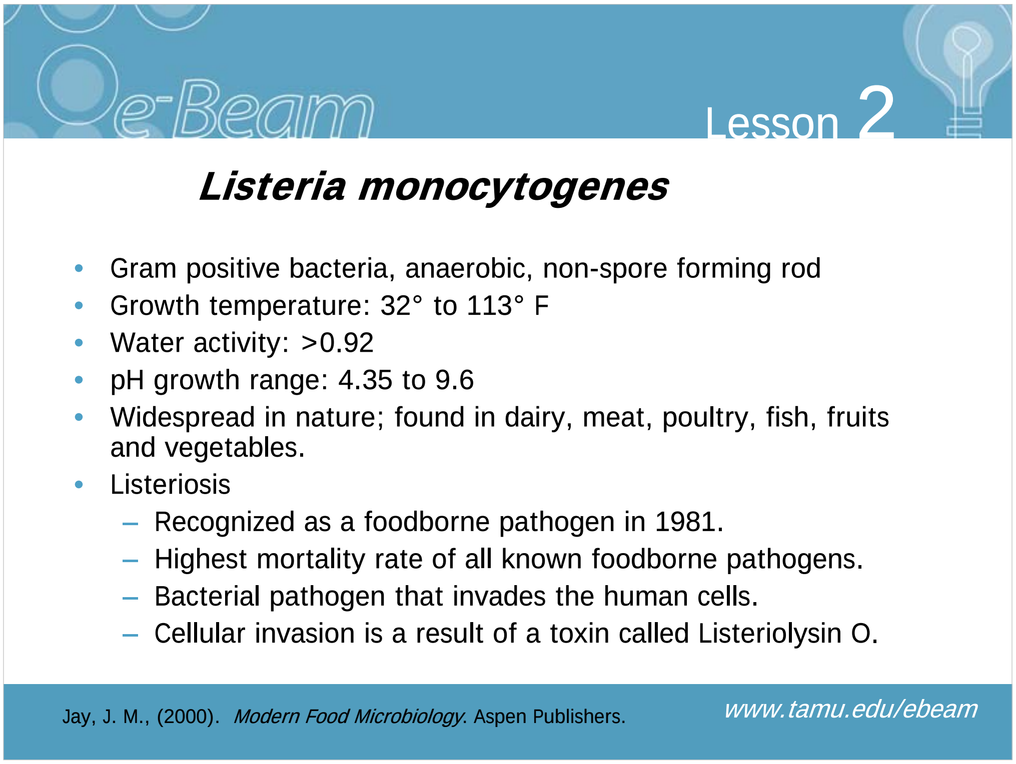
E.coli O157:H7 is a particularly troublesome pathogen. It has a very low infective dose, as few at 10 (10^1) live *E. coli* cells may cause acute symptoms. *E.coli* was implicated in an outbreak in the Northwest in apple cider. Even though Apple cider has a pH low enough to prevent pathogen growth, bacterial cells survived in the unpasteurized apple cider and caused illness in several individuals.

Consequently, the FDA has passed the juice HACCP, or Hazard Analysis Critical Control Point, regulations.



As you can imagine, like other microbes that naturally exist in food production environments, controlling *E. coli 0157:H7* falls into the hands of everyone and every segment of the food chain.

As a matter of fact, since 2000 consumers have demanded up to 20 million pounds of irradiated ground beef per year as a result of *E. coli O157:H7* concerns.



Like *Salmonella*, *Listeria* is a pathogen of concern in many fresh-cut produce products.

Listeria's ability to survive in moist refrigerated processing and storage environments makes it difficult to combat.

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Listeria monocytogenes (*Continued)*

- *Listeriolysin O*
  - *Listeriolysin O* causes pores to form in the intestine (epithelium) and cells of the infected immune system whereby Listeria gains entrance to those cells.
  - Manifestations of the Disease causes 1,600 cases and 415 deaths annually according to FDA/CFSAN.
    - Abortion
    - Neonatal meningitis
    - Adult Listeriosis
    - Gastroenteritis

Jay, J. M., (2000). *Modern Food Microbiology*. Aspen Publishers.

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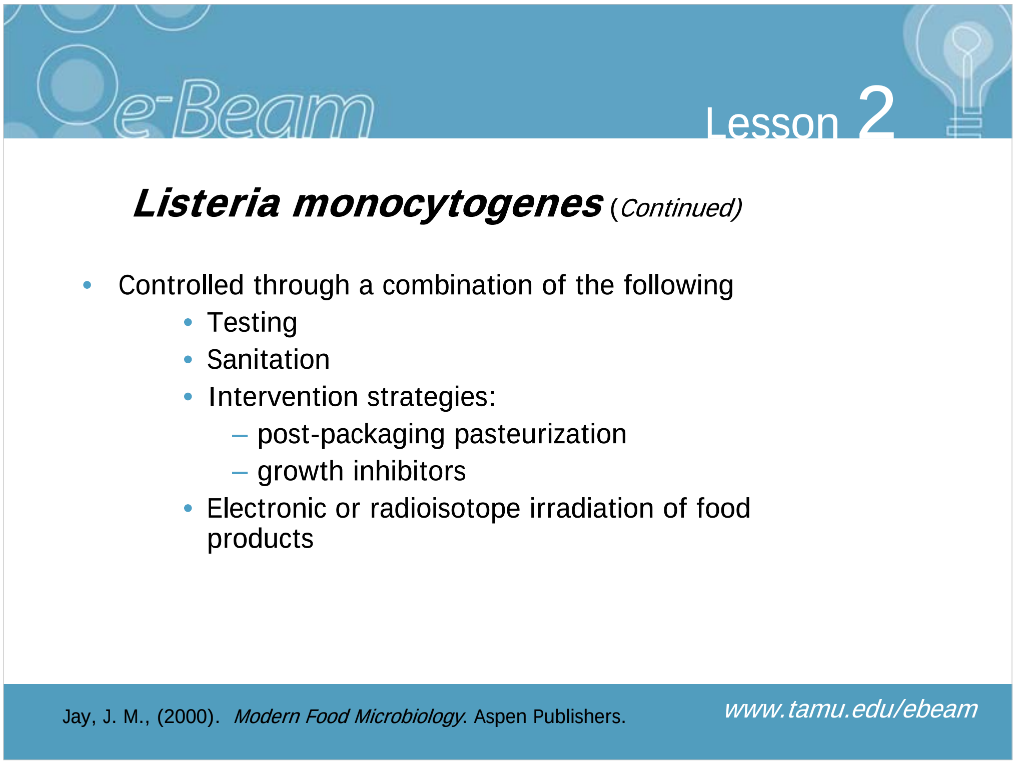
Listeria monocytogenes affects the most vulnerable victims, pregnant women and unborn children.

This pathogen crosses the placental membranes and infects the unborn child. Pregnant woman may abort the fetus or deliver a severely ill child. Most children born with *listeriosis* do not survive.



Processing facilities must have strategic testing and sanitation regimens in their daily routine. Some (meat processors) have implemented heat pasteurization and experimented with irradiation as well as other intervention strategies. It is difficult to remove from a food processing plant once the bacterium have been established.

Lesson 2

Clostridium botulinum

- Gram positive, anaerobic bacteria, spore-forming rod
- Has been found in soil, marine and lake sediments, feces, carcasses of dead animals, foil-wrapped potatoes, rotting vegetation and food.
- Heat resistant spores are widely distributed
- Must prevent growth and toxin formation
- Botulism
  - First identified in 1896
  - Growth produces potent neurotoxins which are some of the most lethal substances known.
  - Toxin binds to nerve endings with symptoms arising 12 to 72 hours after consumption.
  - Symptoms include: nausea, vomiting, fatigue, dizziness, headache, dryness of skin, mouth and throat, paralysis of muscles, double vision, and difficulty breathing.

Jay, J. M., (2000). *Modern Food Microbiology.* Aspen Publishers.

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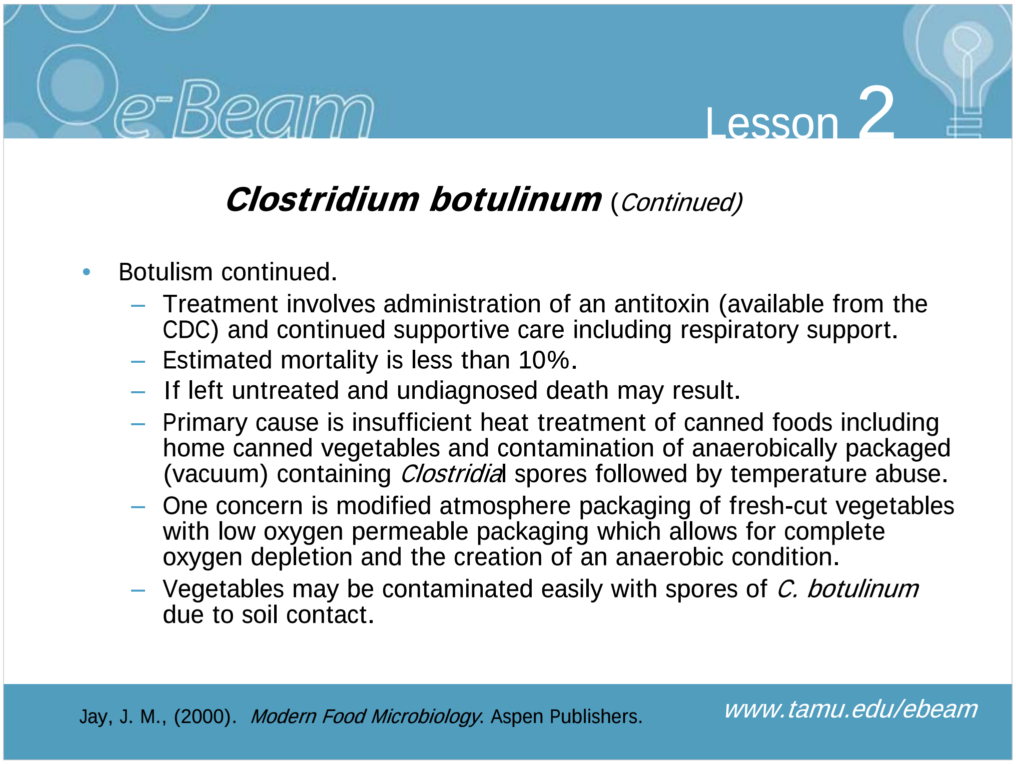
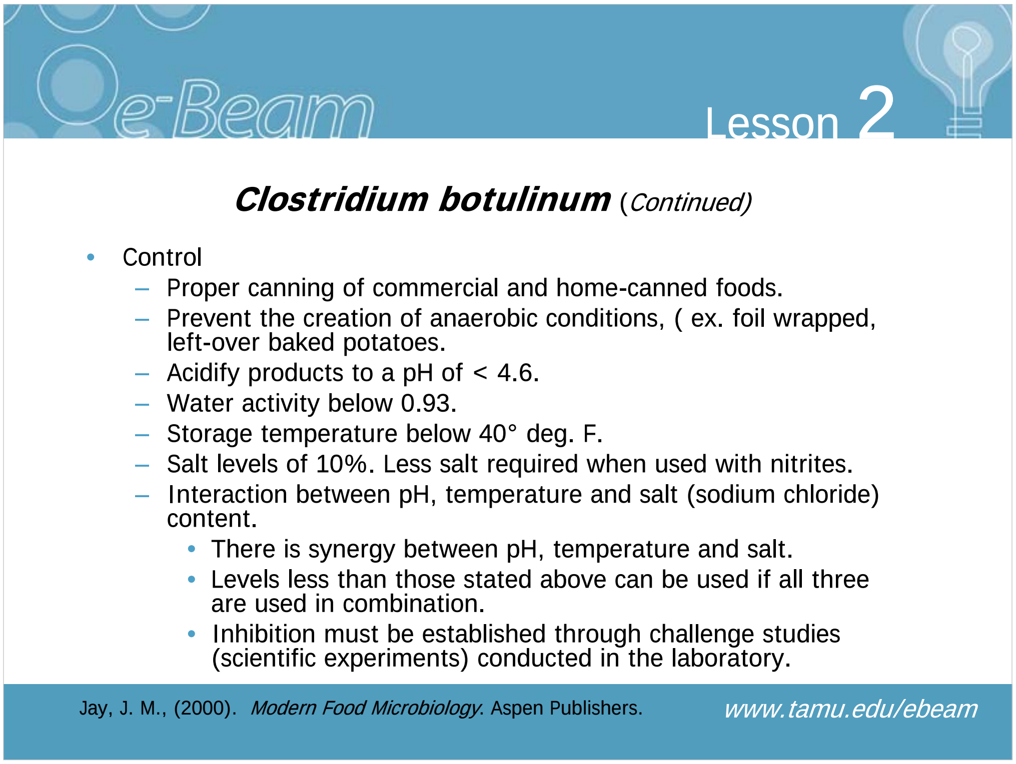
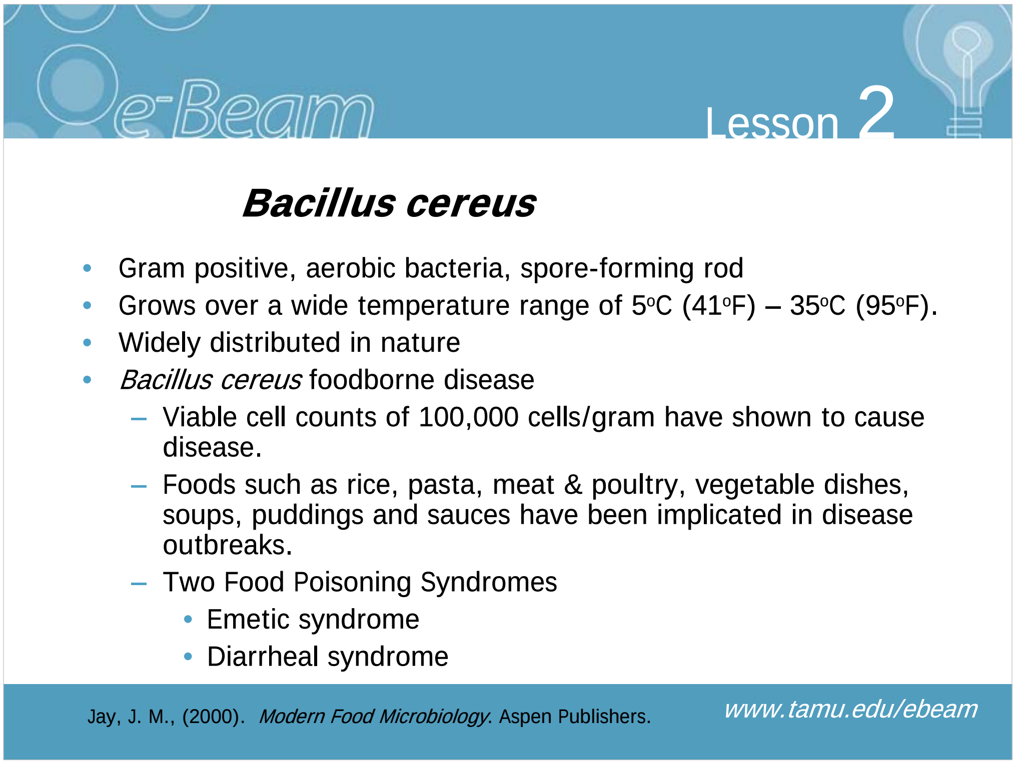
Although some people use one type of neurotoxin (Botox) produced by this bacterium for medical or cosmetic purposes, *Clostridium botulinum* is host to some serious food safety implications.



Fresh-cut servings and family size bags of vegetables with modern, low-oxygen packaging create an attractive anaerobic atmosphere for this organism.



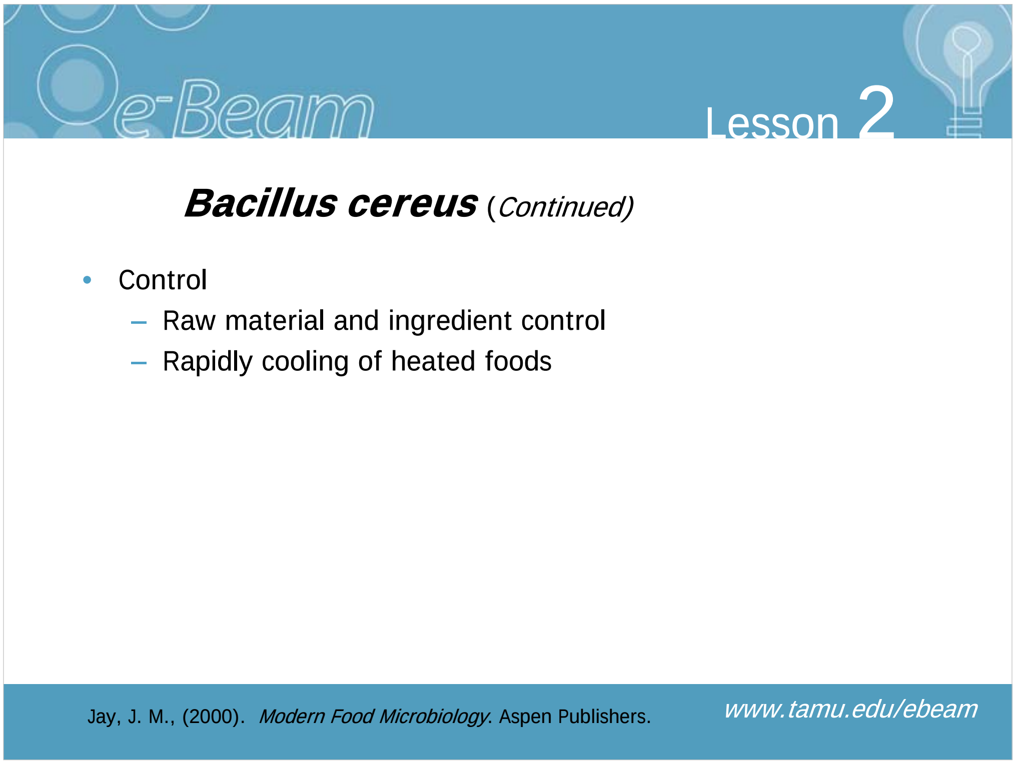
There are several proven control methods.



Bacillus cereus food poisoning doesn't receive much media attention. This organism is widely distributed in nature and its presence at low levels in foods is not a reason for concern. Most incidences of *Bacillus* outbreaks involve inappropriate holding times and temperatures. Low levels of this organism in foods are not a concern as long as appropriate holding times and temperatures are observed. Most outbreaks occur in restaurants and food service establishments.

Two food poisoning syndromes exist for Bacillus cereus, the emetic and diarrheal. In the emetic syndrome toxin is produced in foods prior to consumption. This syndrome causes nausea, vomiting, and diarrhea a short time after consumption (1 to 5 hours). Symptoms last from 6 to 24 hours.

The diarrheal syndrome involves the ingestion of cells which contain and then produce toxin in the small intestine. Abdominal pain, diarrhea, and occasionally nausea sets in 8 to 16 hours after consumption. The symptoms usually last 12 to 24 hours.



This bacterial pathogen is controlled by controlling its source in raw materials and by cooling products rapidly (from 130 to 50 deg. F. in 4 hours).

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Hepatitis A Virus

- Most prominent foodborne virus
- Incubation period is 15 to 45 days
- Fecal-oral route is the mode of transmission
- From 1983 through 1987, there were 1,067 cases of *Hepatitis A* resulting in 1 death
- October-November 2003, *Hepatitis A* outbreaks linked to Mexican green onions affecting people in Tennessee, North Carolina and Georgia with 530 confirmed cases and three deaths.

Jay, J. M., (2000). *Modern Food Microbiology.* Aspen Publishers.

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Hepatitis A is the most prominent foodborne virus.

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Noroviruses

- 42% of nonbacterial outbreaks reported to CDC between 1976 and 1980 were found to be Norwalk virus.
- > 23 million cases each year (Mead, J. Infectious Diseases, 2002)
- More resistant to chlorine disinfection than the *Hepatitis A* virus.
- Can withstand 5-6 ppm of chlorine.
- Incubation period is 18 to 48 hours
- Symptoms
  - nausea
  - vomiting
  - diarrhea
  - abdominal cramps

Jay, J. M., (2000). *Modern Food Microbiology.* Aspen Publishers. *www.tamu.edu/ebeam*

Noroviruses have become common on cruise ships in recent years.

e-Beam Lesson 2

Foodborne Parasites

- Foodborne parasites that can be contracted by consuming certain foods are protozoans.
- Protozoans are organisms similar to algae and flagellate fungi.
- Parasites of particular concern in fresh-cut produce are:
  - *Giardia lamblia*
  - *Cyclospora cayetanesis*
  - *Cryptosporidium parvum*

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Parasites of particular concern in produce include *Giardia*, *Cyclospora* and *Cryptosproidium* all of which have received media attention in recent years due to outbreaks.

e-Beam Lesson 2

Giardiasis

- Caused by *Giardia lamblia*.
- Exists in environmental waters.
- Organism is resistant to chlorine levels used in the water supply.
- Cysts are the primary form in waters.
- Muskrats and beavers harbor the organism and are the major source for water contamination.
- Cysts begin active growth in the upper small intestine and begin to absorb nutrients.
- Symptoms may last from several months to a year.
  - Abdominal cramps, diarrhea, abdominal distention, malnutrition & weight loss
- Vegetables are contaminated from use of contaminated water or from vegetables being handled by asymptomatic carriers.

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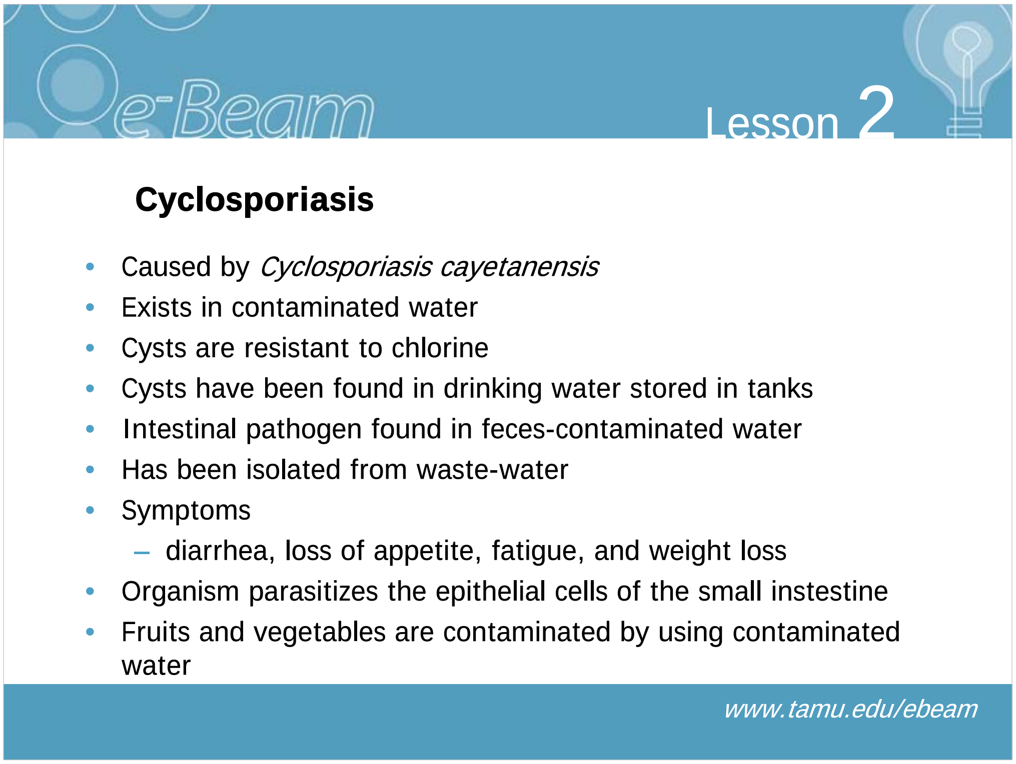
Unlike the viral or bacterial pathogens, parasites are very large and can be detected by direct means using concentrating and staining techniques. Also, unlike bacterial or viral pathogens, the actively growing cell is not consumed, but rather a resting form called the cyst is consumed.

A cyst is a dormant form assumed by the parasitic microorganism in which the organism is enclosed in a walled membranous structure serving a protective function for the resting organism.

The *Giardia* organism begins to actively grow within the host by absorbing nutrients from the upper intestinal tract. Upon consumption, the organism has an incubation period of 7 to 13 days. Carriers may be symptomatic or asymptomatic. One route of contamination for vegetables involves the fecal-oral route in which the handler is asymptomatic and, through poor hygiene, contaminates the produce they handle. The cysts appear in the stool 3-4 weeks after infection. Vegetables may also be contaminated by water that is contaminated. *Giardia* is resistant to normal levels of chlorine used in drinking water.

The US Food and Drug Administration considers Giardiasis the most frequent cause of non-bacterial diarrhea in North America. Giardiasis is present throughout the population, but its prevalence is higher in children. The disease is common in daycare centers where diapering is performed.



Contaminated water may be the origin of a condition where the epithelial cells of the small intestine are parasitized by the *Cyclosproriasis* organism.

Lesson 2

Cryptosporidiosis

- Caused by *Cryptosporidium parvum*
- Present in environmental waters
- Cysts invade host cells where sexual reproduction occurs
- Chlorine and ozone are ineffective at destroying organism
- Human cryptosporidiosis may be acquired by person-to-person contact, person-to-animal contact, or through contaminated food or water.
- Symptoms
  - profuse diarrhea, abdominal pain, low-grade fever, & vomiting.
- Fruits and vegetables are contaminated by use of contaminated wash water or through an infected food-handler through the fecal-oral route.

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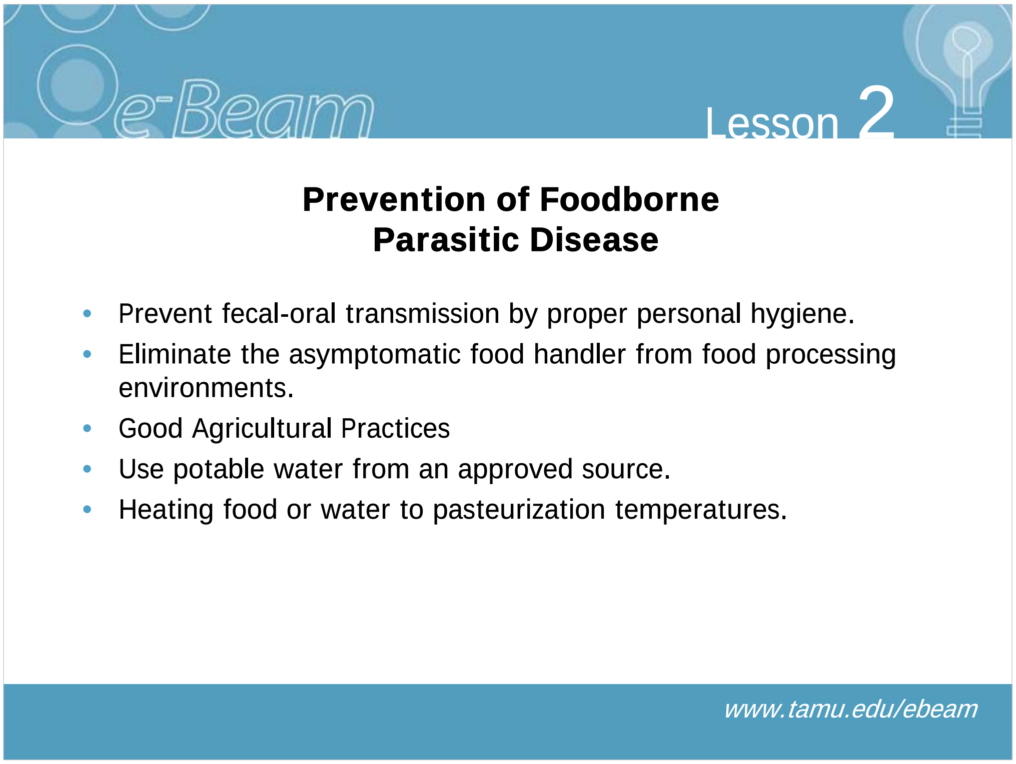
Human cryptosporidiosis may be acquired by person-to-person contact, person-to-animal contact, or through contaminated food or water.



Although parasites can be formidable opponents in our efforts to reduce or eliminate foodborne disease, there are ways to reduce the likelihood of contracting a parasitic disease. Proper hygiene is of the utmost importance in reducing not only parasitic foodborne disease, but ALL foodborne disease. Screening food plant employees for signs of illness and creating a work-place atmosphere where workers will alert management of these health conditions is essential. Good agricultural practices in which animal wastes are eliminated as sources of fertilizer and adequate toilet and hand-washing facilities for harvesters and packing house employees is provided will add another hurdle in the effort to prevent foodborne disease. Filtration of wash-water at a level of less than 1 micron will eliminate the cysts from the water. Finally, pasteurization is the most effective means of preventing foodborne disease. Not all fruits and vegetables are able to be heated, but many foodborne disease outbreaks can be eliminated with proper heating.

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Number of Foodborne Disease Outbreaks and Illnesses for Selected Foods in the U. S. for 1988–1992 and 1993-1997

Food Type	1988 - 1992				1993 - 1997			
	Outbreaks		Illnesses		Outbreaks		Illnesses	
	#	%	#	%	#	%	#	%
Fruits & Vegetables	64	2.64	2,448	3.16	66	2.40	11,638	13.52
Beef	63	2.60	2,085	2.69	66	2.40	3,205	3.72
Chicken	40	1.65	40	1.65	30	1.10	1,113	1.29
All Food Types	2,424		77,373		2,751		86,058	

Centers for Disease Control, MMWR, October 25, 1996/Vol.45/No.SS-5
Centers for Disease Control, MMWR, March 17, 2000/Vol. 49/No.SS-1

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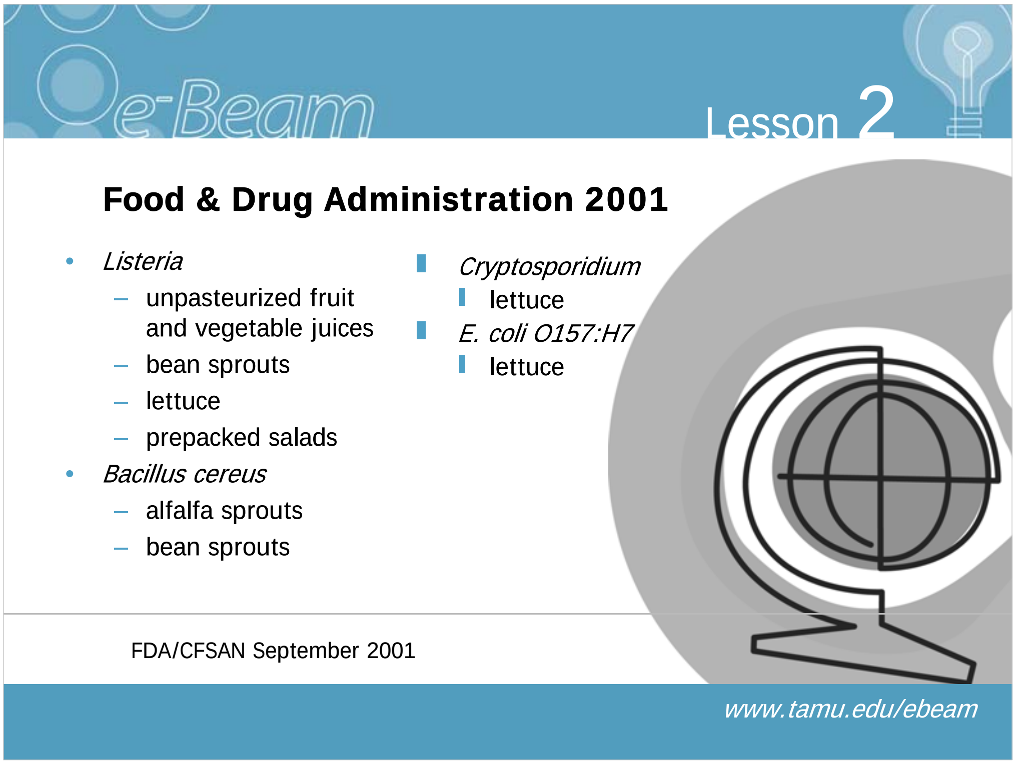
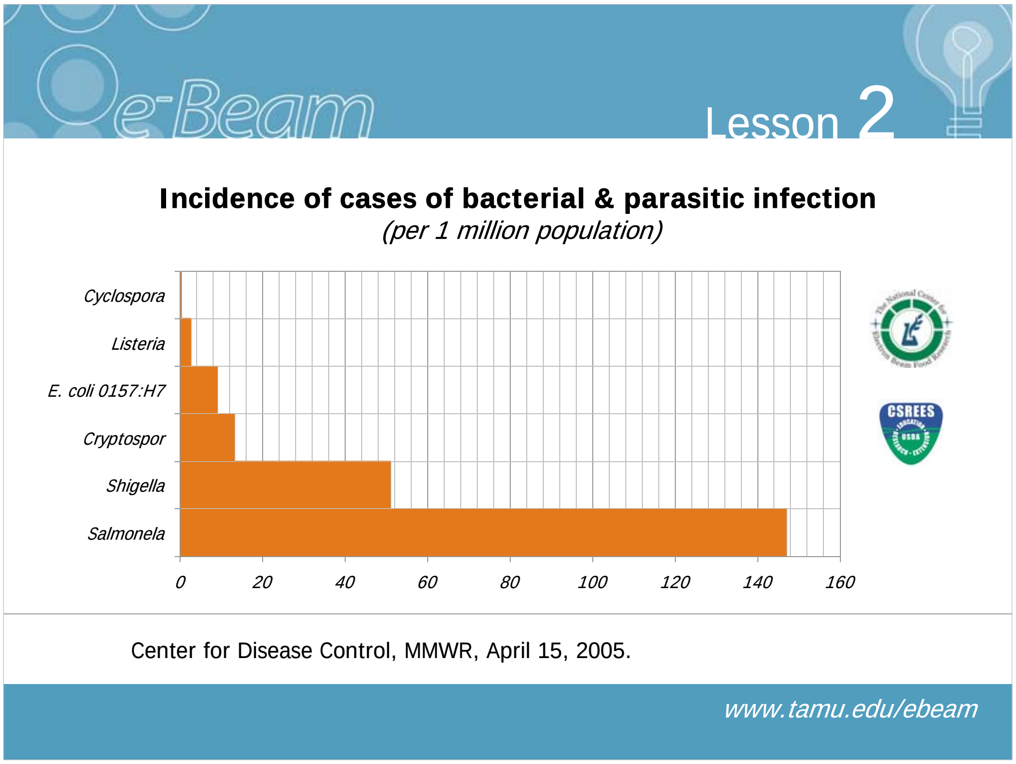
Food inspection has focused on reducing the incidence of foodborne disease associated with meat and poultry. The CDC estimates that only 1 – 5 % of foodborne disease cases are reported. In 1996 and 2000,the Centers for Disease Control released the number of reported foodborne diseases for selected foods for two five-year periods, 1988 – 1992 and 1993 – 1997. As you can see, fruits and vegetables led the list for foodborne disease for both time periods.



In 2001, the Center for Food Safety and Applied Nutrition at the Food and Drug Administration conducted an exhaustive survey of fresh-cut produce implicated in foodborne disease outbreaks. A sampling of the products implicated and their associated pathogen is listed above.

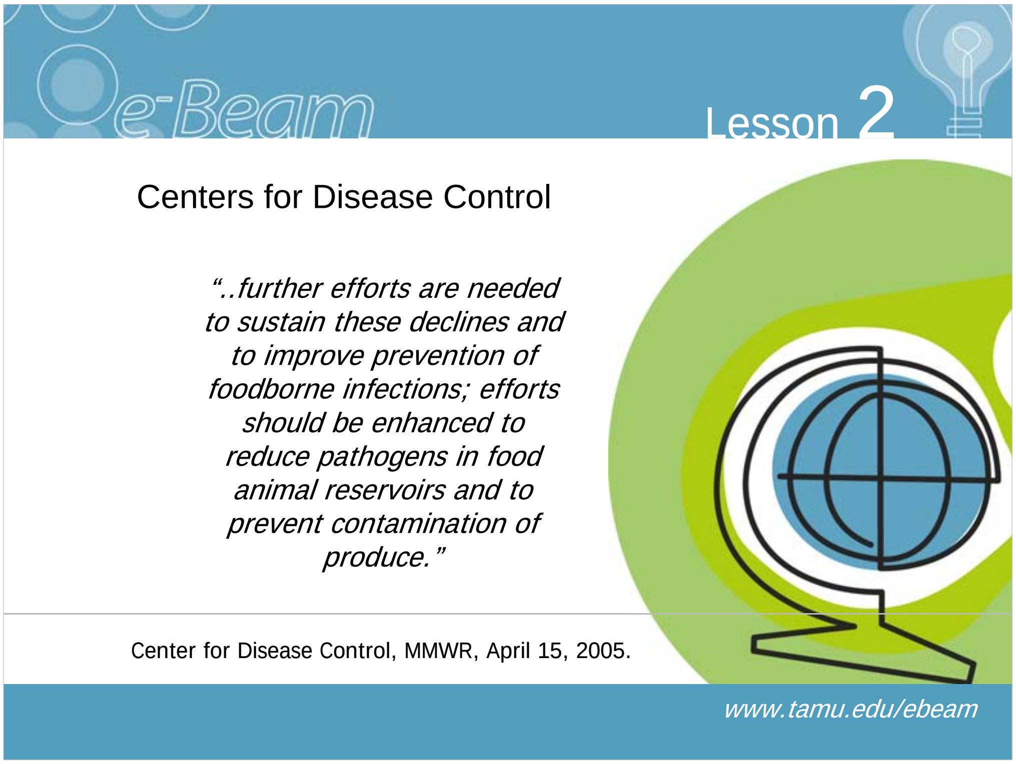
FDA evaluated the preventive control measures for reducing the incidence of foodborne disease associated with fresh-cut produce. Results show there is work yet to be done.



In April, 2005, the Centers for Disease Control released preliminary data for laboratory diagnosed cases of infections.

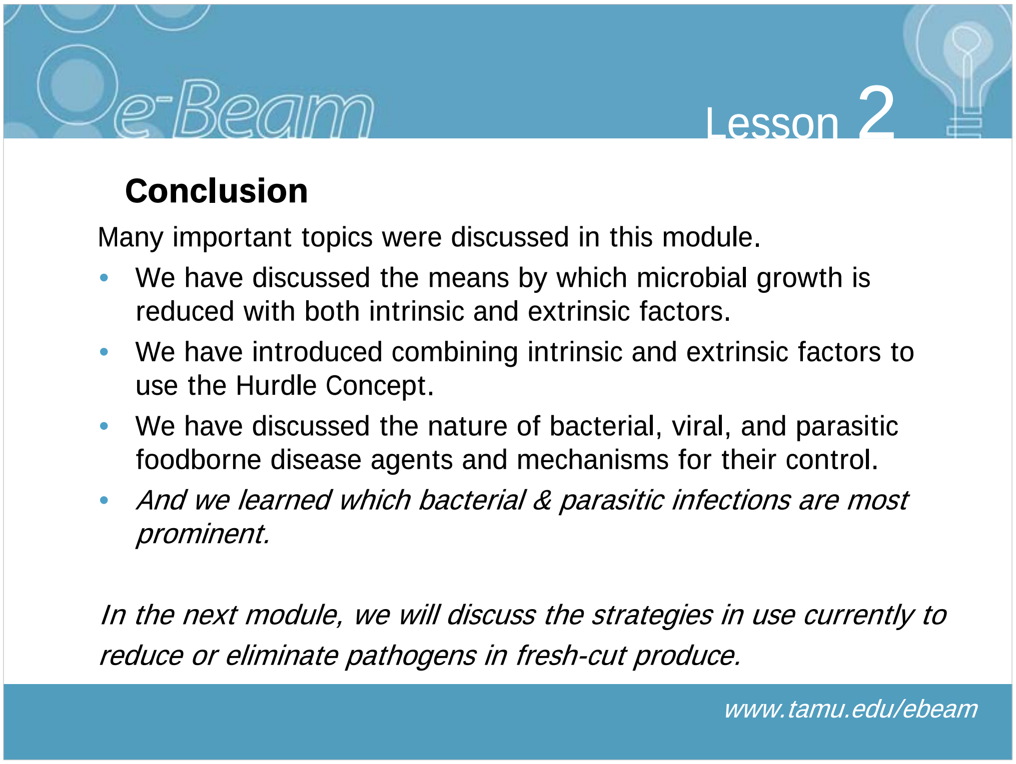
Salmonella still leads the list as the greatest cause of foodborne disease in the United States.

With all the effort placed on the reduction of pathogens using antimicrobial strategies, are we making progress?



In this report, CDC reported a 40% decline in *Cryptosporidium*, a 40% decrease in *Listeria*, a 42% decrease in *E. coli* O157:H7, and yet only an 8% reduction in *Salmonella*.

To continue the current declines and improve on the decline in *Salmonella*, the CDC made the above recommendation.



Many important topics were discussed in this module.

We have discussed the means by which microbial growth is reduced with both intrinsic and extrinsic factors.

We have introduced combining intrinsic and extrinsic factors to use the Hurdle Concept.

We have discussed the nature of bacterial, viral, and parasitic foodborne disease agents and mechanisms for their control.

And we learned which bacterial & parasitic infections are most prominent.

In the next module, we will discuss the strategies in use currently to reduce or eliminate pathogens in fresh-cut produce.