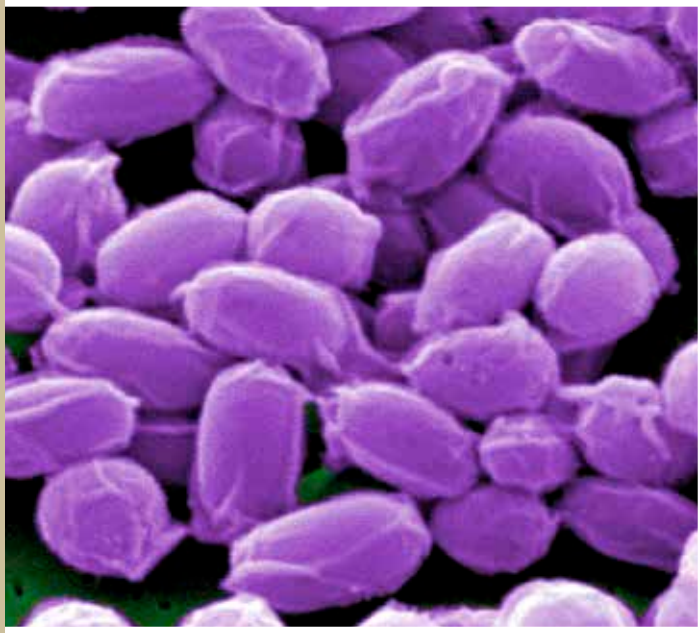
United States
Department of
Agriculture
Food Safety and
Inspection Service

Introduction to the Microbiology of Food Processing



August 2012

Small Plant News Guidebook Series

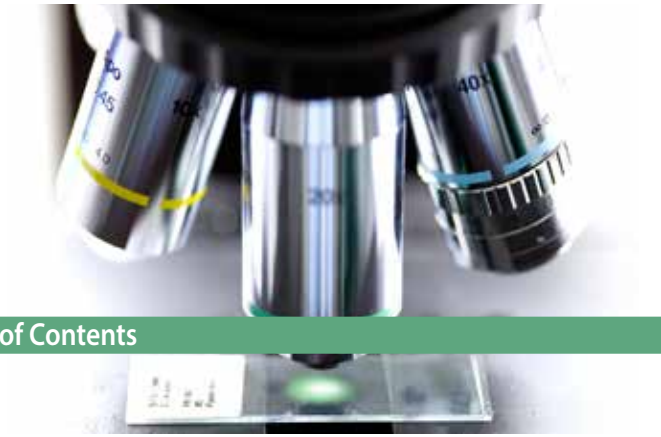
Small Plant News is a four-page, four-color newsletter published by the U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS). It is targeted to small and very small Federal- and State-inspected establishment owners and operators who produce meat, poultry, and processed egg products.

Small Plant News's mission is to support the "FSIS' Strategic Implementation Plan for Strengthening Small and Very Small Plant Outreach" by providing pertinent information for plant owners and operators so they can produce safe food and, ultimately, ensure the success of their livelihoods.

The newsletter strives to do this through:

- ✔ Informing and educating small and very small plant owners and operators on FSIS news with current and meaningful information in an easy-to-read format.
- ✔ Assisting plant owners and operators in incorporating FSIS rules and regulations into their daily operational practices with "plain language" information.
- ✔ Fostering small and very small plants' ability to stay in business and produce the safest food by providing essential tips that will encourage the highest sanitation standards, paperwork compliance, and cost-saving measures.
- ✔ Honoring FSIS' obligations to small and very small plants by providing a mechanism that increases two-way dialogue between plants and the Agency.

Back issues of *Small Plant News* are available on FSIS' Web site at ***www.fsis.usda.gov***. Or you may call the Small Plant Help Desk at (877) 374-7435 to order back copies.



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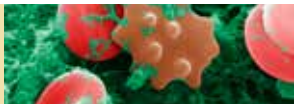


Introduction

Microbiology refers to the study of microorganisms. As the name suggests, microorganisms are organisms that are so small they can only be seen using a microscope. Bacteria, fungi (such as yeasts and molds), protozoa, viruses, algae, and some parasites are all types of microorganisms. Some people also list prions as microorganisms even though they are proteins and not actually organisms. (You may remember that prions are the cause of bovine spongiform encephalopathy [BSE] and its human variation, Variant Creutzfeldt-Jakob Disease [vCJD].)

Microorganisms are all around us, and they are capable of surviving in a wide array of environmental conditions. They are a necessary part of our world and perform a variety of useful functions. Here are some examples:

- **Fermentation**

  Fermentation, the conversion of carbohydrates into sugar and alcohol, is required for the production of beer, wine, many types of cheeses and breads, and some sausages.

- **Digestion**

  Microorganisms help people digest fruits and vegetables, just as they help animals digest plants.

- **Provide vitamins**

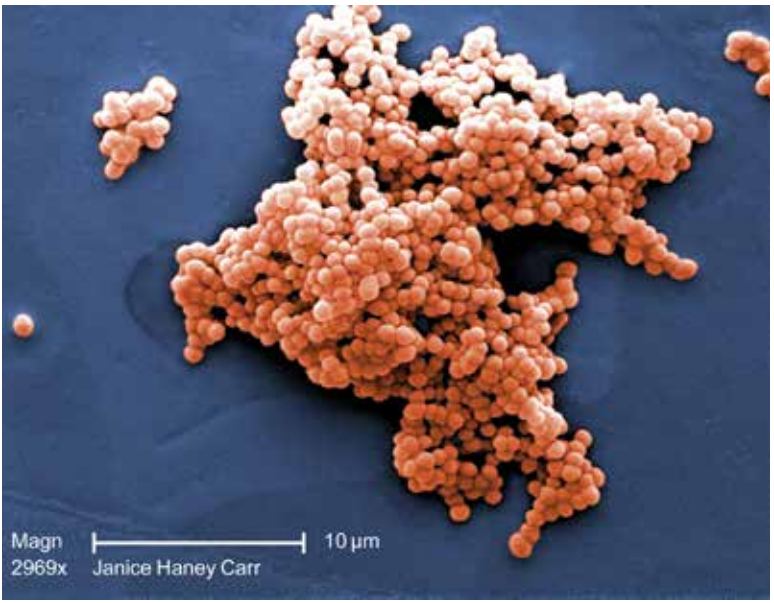
  Some microorganisms synthesize the vitamins we need to stay healthy.

- **Recycling**

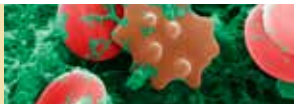
  There are other types of microorganisms that have the ability to break down organic matter (material containing carbon that comes from what used to be a living organism) and return it to the earth in a recycling process to form food for plants, which in turn provide food for animals. This process of breaking down organic matter is part of what we normally think of as spoilage. All raw foods contain microorganisms that will eventually spoil and break down the food. Without such microorganisms, the earth would accumulate dead animals, plants, and other non-decayed matter.

Unfortunately, microorganisms also can be detrimental. They are the cause of many diseases in humans, animals, and plants. Disease-causing microorganisms are called pathogens. A pathogen, or the substances it produces, must invade the human, animal, or plant body to cause illness.

While many diseases are transmissible from person to person or from animals to humans, only a few are transmitted through foods. Diseases that are caused by eating food are usually referred to as food poisoning or foodborne illnesses. This guidebook serves as a broad and very basic introduction to the microorganisms associated with food production, and in particular, bacterial pathogens. FSIS hopes it will assist you, the small and very small plant owner and operator, with your understanding of the microorganisms responsible for foodborne illness and, thus, enhance your ability to use microbial control interventions more effectively in your operations.



C. streptococcus sp



Food Intoxication Versus Food Infection

Microbial foodborne illness is often divided into two broad categories: food intoxication and food infection.

Food intoxication occurs when the pathogenic microorganism secretes a toxin in the food. Eating the food that contains the toxin disrupts a particular target, such as the gastrointestinal tract or the nervous system. The symptoms of intoxication vary from bouts of vomiting and diarrhea to severely disrupted muscle function, as with botulism.

Food infection occurs when the microorganism multiplies in food until it reaches the minimum infective dose (MID), which is the number of microorganisms needed to cause illness in humans. When the food is eaten, the microorganism acts directly on the intestines. In some cases, the microbes infect the surface of the intestine; in others, they invade the intestine and other body structures. Most food infections result in some degree of diarrhea and abdominal distress. Food infection also can result in toxin production, causing the same disease signs. However, in food infection, microbes growing in the infected tissue release the toxins, unlike food intoxication, where the toxins are already present in the food.

The MID varies for different pathogens and can range from as few as 10 cells for the *Shigella* species (and possibly *Escherichia coli* O157:H7) to as many as 100,000 cells for *Staphylococcus aureus*. Some of the factors that influence the infections include:

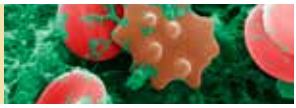
- The immune status of the host—immunodepressed or immunocompetent.
- How efficient the pathogen is at attaching to, and penetrating, the target tissues.
- The number of pathogenic organisms entering the body.

Significant Microorganisms in Food Production

Microorganisms such as molds, yeasts, and bacteria can grow in food and cause spoilage. Bacteria also can cause foodborne illness. Viruses and parasites, such as tapeworms, roundworms, and protozoa, can cause foodborne illness, but they are not capable of growing in food and do not cause spoilage.

The following is a list of pathogens and infectious agents of public health concern. This list is not exhaustive, but contains most of the foodborne pathogens that affect meat, poultry, and processed egg products.

- Bacteria
  - *Bacillus cereus* (*B. cereus*)
  - *Brucella* species *(Brucella* spp*)*
  - *Campylobacter* spp
  - *Clostridium botulinum* (*C. botulinum*)
  - *Clostridium perfringens* (*C. perfringens*)
  - *Escherichia coli* O157:H7 (*E. coli* O157:H7) and other Shiga-toxin-producing *E. coli* (STEC) (including O26, O45, O103, O111, O121, and O145)
  - *Listeria monocytogenes* (*L. monocytogenes*)
  - *Salmonella* spp
  - *Shigella* spp
  - *Staphylococcus aureus* (*S. aureus*)
  - *Yersinia enterocolitica* (*Y. enterocolitica*)



- Viruses
  - Hepatitis A and D
  - Norovirus
  - Rotaviruses
- Tapeworms
  - *Taenia* spp
- Roundworms
  - *Trichinella* spp
- Protozoa
  - *Toxoplasma* spp
  - *Sarcocystis* spp

The U.S. Centers for Disease Control and Prevention (CDC) reports that the most common foodborne illnesses for which an organism has been identified are those caused by the bacteria *Campylobacter, Salmonella, L. monocytogenes*, and *E. coli* O157:H7 and the norovirus.

Foodborne Pathogens

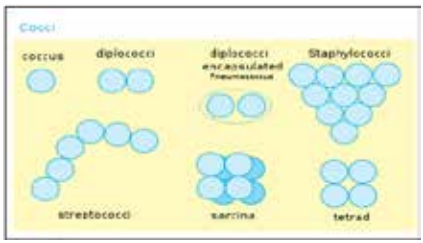
Bacteria

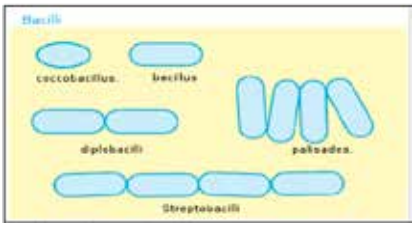
General Information on Bacteria

Bacteria are the most important and troublesome of all the microorganisms for the food processor. Bacteria are single-celled living bodies. Varying in length from 1/25,000 to 1/1,000 of an inch, they are among the smallest living creatures known.

There are many different ways to classify and group microorganisms, such as microscopic appearance; materials they can use as foods; byproducts resulting from the breakdown of these foods; tolerance to oxygen; growth temperatures; resistance to destructive agents, such as heat and chemicals; ability to perform certain biochemical reactions in the laboratory; and possession of certain genetic sequences.

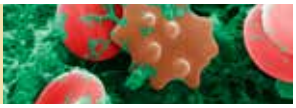
Viewed with a microscope, bacteria appear in several shapes or forms, but are primarily either round in shape (called "cocci") or rod-shaped (called "rods" or "bacilli").





Spore Forming and Non-Spore-Forming Bacteria

Spores are at dormant or resting stage in the normal growth cycle of some bacteria. The primary function of most spores is to ensure the survival of the organism through periods of environmental stress. Spores have been compared to plant seeds because they will "sprout" (germinate) and grow when conditions are suitable. When a bacterial spore germinates, it is the same organism continuing its growth process. The process of spore formation is called sporulation.



Bacteria can be placed into two groups based on their ability to form spores: spore formers and non-spore formers. As you may have guessed, spore formers can form spores, and non-spore formers cannot form spores. The four major spore-forming bacteria are *C. botulinum*, *C. perfringens*, *B. cereus*, and *Bacillus anthracis* (*B. anthracis*). These bacteria are normally present everywhere in the environment, which can make them difficult to control in a food-processing facility.

Cells of non-spore formers, cells of spore formers that have not formed spores, and cells that have sporulated are referred to as "vegetative cells." Vegetative cells generally have little resistance to heat, drying and other unfavorable conditions. However, bacterial spores in general are extremely resistant to heat, cold and chemical agents. For example, some bacterial spores can survive in boiling water (212 °F or 100 °C) for more than 16 hours, but vegetative cells (same organisms in the vegetative state and the non-spore-forming bacteria) cannot. Generally, spores that resist heat also are highly resistant to destruction by chemicals. There are bacterial spores that can survive more than 3 hours in sanitizing solutions normally used in a food-processing plant. These same sanitizing agents easily destroy vegetative cells.

Because bacterial spores can survive in unfavorable conditions, they can be present in food before, during, and after lethality treatment is applied. This is why the time it takes to heat and/or cool product during food processing is so important. If the food product is not heated or cooled appropriately – that is, the product stays for too long in a temperature range that is favorable to bacterial growth—the spores will change to a vegetative state and start reproducing. This is called outgrowth of the spores. The bacterial cells can reproduce to reach the MID and cause a food infection, or produce a sufficient amount of toxins to create a food intoxication.

Bacterial Toxins

Toxins are substances produced by living cells or organisms that are capable of causing disease when they come in contact with, or are absorbed by, body tissues. They can vary greatly, both in the severity of effect (ranging from minor to fatal) and how easily they can be destroyed. One way to categorize toxins depends on where they affect the body:

- Enterotoxins

  Affect the gastrointestinal tract, causing vomiting, diarrhea, gastrointestinal distress and/or pain.

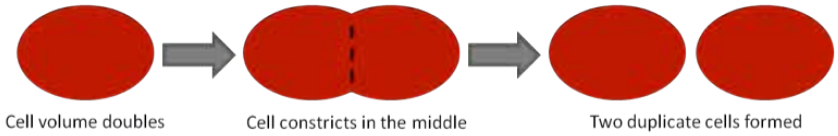
- Neurotoxins

  Affect the nervous system, causing dizziness, blurred or double vision, the inability to swallow, skeletal muscle weakness, and/or flaccid paralysis.

Depending on the bacteria producing the toxin, it can be a byproduct of the sporulation process or a byproduct of normal cell metabolism. Toxins that are already present in the food product are often referred to as "preformed toxins." If there is enough of the preformed toxin present, just eating the food will cause illness. Some toxins are extremely heat resistant and may pose a hazard even if the food is properly cooked. Toxin-producing bacteria include *S. aureus*, *B. cereus*, *C. botulinum*, and *C. perfringens*.

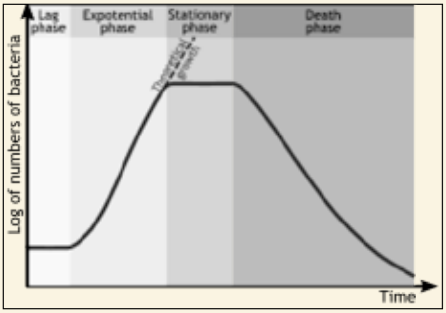
Bacterial Cell Reproduction

Bacteria reproduce by division, which is what microbiologists call fission. When a bacterial cell is ready to divide, the cell material gradually increases until its volume is almost doubled. The cell then constricts in the middle. This constriction deepens until the cell contents are held in two distinct compartments separated by a wall. These two compartments finally separate to form two new cells, which are duplicates of the former cell and each other.



As the individual cells reproduce on a Petri dish, they form a group known as a colony. When we think about measuring bacteria, we usually think about colonies rather than individual bacterial cells. Since the reproduction of bacteria increases their numbers, the size of the colony grows. This is why bacterial reproduction is sometimes referred to as growth. "Bacterial growth" refers to an increase in the number of bacteria present, not to an increase in the size of the individual bacterial cells.

The following graph shows the four phases of bacterial growth (reproduction):



- Lag phase-Occurs when a bacterial population first enters an environment that allows growth.
  - Growth is slow because the bacterial cells are adapting to their new environment.
  - Most variable of the phases because the amount of time that is needed to adapt depends on factors, such as the characteristics of the bacteria and the environment.
  - The length of the phase affects how quickly the MID of pathogenic bacteria is reached.
  - The length of the phase affects whether the interventions that your plant has in place are sufficient to control the numbers of pathogenic bacteria.
- Log phase or exponential phase-Occurs when the number of cells increases exponentially in a favorable environment.
  - Numbers of bacterial cells will double every 20 to 30 minutes.
    - The rate at which bacterial cells reproduce is known as growth rate (growth rate = k).
    - The time it takes for bacterial cell numbers to double is known as generation time (generation time = g).
  - Nutrients in the environment are used up quickly until one of the nutrients is gone and growth slows down.

- Stationary phase-The phase where the rate of bacterial growth equals the rate of bacterial death.
  - The reproduction of cells slows down because nutrients are used up in the log phase.
  - The increase of byproducts from bacterial growth can inhibit further growth.
- Death phase-Reproduction rate is lower than the death rate.
  - More bacterial cells are dying than reproducing.
  - There is a net loss of bacterial cells capable of reproduction.
  - Some bacteria can form resistant spores and survive this phase.

Pathogenic Bacteria of Importance

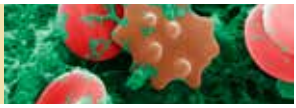
Let's take a closer look at significant bacteria that you should address in your operation.

Salmonella species (*Salmonella* spp.)

Transmission	fecal-oral ingestion
Source	beef, poultry, milk, or eggs
Immediate symptoms	nausea, vomiting, abdominal cramps, diarrhea, fever, and headache
Long-term consequences	may follow 3-4 weeks after the onset of acute symptoms
Onset time	ranges from 6-48 hours
MID	as few as 15-20 cells
Length of disease	averages from 1 to 7 days

In food processing, *Salmonella* infection is of concern in raw poultry, swine, and ready-to-eat products. There are specific regulatory requirements for reduction of *Salmonella* in ready-to-eat products, raw ground beef, and raw beef components that are intended for grinding.

Salmonella is a rod-shaped, generally motile, non-spore forming, Gram-negative bacterium. It grows at 41-117 ˚F (5-47 ˚C), at a pH as low as 4.2,



with or without air, and at a water activity (measure of available water) greater than 0.94 (a_w >0.94). The optimum growth temperature is at the human body temperature (98.6 °F). *Salmonella* remains viable for a long time in frozen foods. All *Salmonella* serotypes (strains) are considered potential human pathogens, but only a few serotypes are associated with foodborne illness (salmonellosis): *Typhimurium*, *Enteriditis*, *Newport*, *Heidelberg*, and *Javiana*. Pathogenic *Salmonella* possess factors that enhance their ability to invade and replicate in the host.

Salmonella is an enteric microorganism associated with the intestinal tract of many animals and, thus, is potentially present in most raw meats. Illness is usually caused by the ingestion of a sufficient number microorganisms which survive digestion and reproduce in the human intestinal tract. Fortunately, *Salmonella* are heat-sensitive and easily destroyed with the mild heat treatments for cooking meat (heating to 145 °F [63 °C]). *Salmonella* also are acid-sensitive and are not good at competing with other bacteria. However, it should be noted that acid resistance can be induced by exposure to mildly acidic conditions (pH 5.5-6.0), resulting in tolerance to extremely acidic environments (pH 3-4). *Salmonella* has not been a problem with fermented sausages when the product is properly fermented using an appropriate starter culture. *Salmonella* not only survives drying, but also becomes more heat-resistant with drying and is more of an issue in non-fermented dried meats, such as jerky, and whole meat cuts, such as dried hams. *Salmonella* remains viable for a long time in frozen foods.

Control mechanisms include temperature control and sanitation prior to processing and the use of wet heat early in the heat process to avoid pre-drying. In non-heated meats, the proper use of high salt and proper curing techniques are most effective.

The prevalence of *Salmonella* in beef, lamb, pork, and poultry carcasses varies greatly. The overall contamination of meat and poultry carcasses with these pathogens depends not only on the numbers of the pathogens on the hair, hide, feathers, skin, and in the intestinal tract of the animals, but also on the degree of cross-contamination occurring from these sources during slaughter and processing.

Salmonella has been isolated from a wide variety of foods, such as spices, milk and dairy products, fish, shrimp, coconut, salad dressing, cake mixes, cream-filled desserts, dried gelatin, peanut butter, and chocolate.

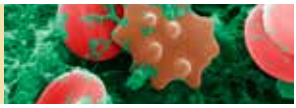
Environmental sources of the organism include water, soil, insects, factory surfaces, kitchen surfaces, and animal feces, to name a few.

Escherichia coli (*E. coli*)

Transmission	fecal-oral ingestion
Source	raw ground and non-intact beef products, raw vegetables, raw milk
Immediate symptoms	severe cramping (abdominal pain) and diarrhea, which is initially watery, but becomes grossly bloody; occasional vomiting; low-grade or no fever
Long-term consequences	Hemolytic Uremic Syndrome (HUS) – More commonly affects children under 10, but may also affect adults and the elderly; characterized by hemolytic anemia, and thrombocytopenia, and kidney failure may follow after a week.
Onset time	approximately 4 days (median)
MID	as few as 10 bacterial cells
Length of disease	average duration of 8 days

In food processing, pathogenic *E. coli* infection is of concern in raw ground and non-intact beef products and dry-cured salami. There are specific regulatory requirements for the reduction of *E. coli* O157:H7 in raw ground and non-intact beef products.

E. coli O157:H7 is a rod-shaped, generally motile, non-spore-forming, Gram-negative bacterium. It generally grows at 45-115 ˚F (7-46 ˚C), at a pH range between 4.4-9, with or without air, and at an a_w >0.95. There are strains of



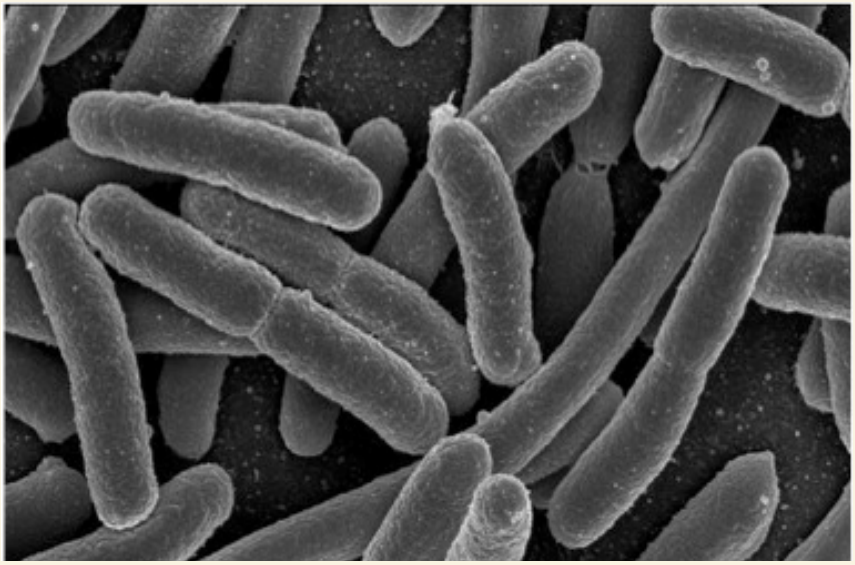
E. coli O157:H7 that can survive in water and possess an unusual tolerance to environmental stress, such as temperature, pH, and dryness.

A minority of *E. coli* serotypes are capable of causing human illness by different mechanisms. The pathogen of primary concern is *E. coli* O157:H7, which is a Shiga-toxin-producing *E. coli* (STECs). Other types of *E. coli*, referred to as non-O157:H7 STECs, also can cause human illness. The strains of most concern are *E. coli* O26, O103, O111, O121, O45, and O145.

E. coli O157:H7 causes hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). All people are believed to be susceptible to HC, but young children and the elderly appear to be the most frequently effected by HUS.

E. coli is a normal inhabitant of the intestines of all animals, including humans; and it serves a useful function in the body by suppressing the growth of harmful bacterial species and by synthesizing appreciable amounts of vitamins.

Although always present, *E. coli* O157:H7 appears to be more prevalent from April through October, peaking in the summer months.



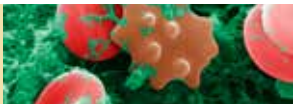
Escherichia coli (E. coli) under a microscope.

Listeria monocytogenes (*L. monocytogenes*) (*Lm*)

Transmission	oral ingestion
Source	ready-to-eat (RTE) hot dogs, deli meats, pâté and meat spreads, fermented raw meat sausages, and raw poultry and meat (all types)
Immediate symptoms	influenza-like symptoms including persistent fever non-invasive form – febrile gastroenteritis invasive form – septicemia, meningitis, encephalitis, intrauterine or cervical infections in pregnant women
Long-term consequences	Not Applicable
Onset time	influenza-like symptoms – may be greater than 12 hours • non-invasive form – probably greater than 18-20 hours • invasive form – may range from a few days to 3 weeks
MID	Unknown. May vary with the strain and susceptibility of the individual.
Length of disease	days to weeks

In food processing, *L. monocytogenes* infection is of concern in ready-to eat (RTE) food-products, particularly hot dogs and deli meats. There are specific regulatory requirements for control of this pathogen in the product and for sanitation in the RTE food-producing facility.

L. monocytogenes is a rod-shaped, non-spore-forming, Gram-positive bacterium. *L. monocytogenes* is a human pathogen that causes listeriosis. It is motile and can grow in cool (temperature range of 32-113 ˚F [0-45 ˚C]) and damp environments, at a pH range of 4.4-9.4, and at a a_w > 0.92.



Some characteristics that make some strains of *L. monocytogenes* hardy include the ability to grow and/or survive in acidic environments (pH 4.39), withstand heat treatments, grow at refrigeration temperatures, and survive in a concentrated salt solution (as high as 30.5 percent at 39.2 °F). Temperatures higher than 170 °F will inactivate the *Listeria* organisms. Food-producing facilities must ensure that RTE products are not recontaminated with *L. monocytogenes* from environmental sources after the bacteria have been inactivated by the cooking process ("lethality step").

L. monocytogenes is found in the environment and can be carried by humans and animals. In the plant, it can be found on equipment, utensils, humans, in water, air flow, etc., and has been isolated at every level of the meat processing chain in slaughter and processing plants. It is of concern in the production of dried meats since it can grow aerobically or anaerobically and can survive dry conditions.

L. monocytogenes can form biofilms, which serve as protective shells. Cells can travel to other sites and start the cycle of biofilm formation at a distance from the original site. Biofilms can form within a few hours or days depending on the number of bacterial cells, available nutrients, surface characteristics, and temperature. Once formed, they can persist for a long time (years), be very difficult to remove, and provide protection from the chemicals used to clean and sanitize surfaces, freezing, drying, high salinity, and heat.

Its presence in the RTE environment can pose a serious problem, especially in the RTE finished product and food contact surfaces. Product flow must be designed to segregate finished products from raw products, and personnel who handle RTE products should be restricted to specific areas to prevent cross-contamination.

In addition to good sanitation and avoiding cross-contamination, *Listeria* can be controlled by a combination of lower pH, high-brine concentration, and lactic acid starter cultures that provide harmless bacteria for *Lm* to compete against.

Generally, listeriosis occurs among the elderly, pregnant women (the outcome upon exposure of the fetus to the pathogen results in spontaneous abortion, stillbirth, or neonatal sepsis), diabetics, people on kidney dialysis, and the immunocompromised (bone marrow transplant patients, cancer patients, etc.).

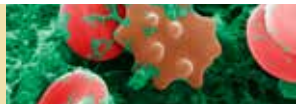
Campylobacter jejuni (C. jejuni) (Campy)

Transmission	fecal-oral ingestion
Source	raw chicken
Immediate symptoms	diarrhea, which may be watery or sticky and can contain blood; fever; nausea; cramping; abdominal pain; headache; and muscle pain
Long-term consequences	*Guillain-Barré* syndrome, a rare disease that affects the nerves of the body; begins several weeks after the diarrheal illness; 1 in every 1,000 reported cases
Onset time	2-5 days after exposure
MID	fewer than 500 cells
Length of disease	generally lasts 7-10 days

In food production, *C. jejuni* infection is of concern in raw poultry products. There are regulatory performance standards for the control of *Campylobacter* on chilled carcasses in young chicken (broiler) and turkey slaughter plants.

Campylobacter jejuni is a Gram-negative, curved, and motile rod. It is a microaerophilic organism, which means it has a requirement for reduced levels of oxygen and requires 3-5 percent oxygen and 2-10 percent carbon dioxide for optimal growth. *C. jejuni* is relatively fragile and susceptible to environmental stresses, such as 21 percent oxygen, drying, heating, disinfectants, and acidic conditions. It can grow at temperatures between 90 and 113 ˚F (32-45 ˚C), at a pH range of 4.9-9.0, and at a_w > 0.98. *Campylobacter* dies off rapidly at ambient temperatures and atmosphere, and grows poorly in food.

The illness caused by *C. jejuni* is called campylobacteriosis, enteritis, or gastroenteritis. It is thought that *C. jejuni* first colonizes the small intestine (jejunum and ileum) and then the colon, producing a heat-labile toxin that may cause diarrhea. It is one of the most common bacterial causes of diarrheal illness in the United States. Campylobacteriosis occurs more frequently in the summer months than in the winter. It lives in the intestines of healthy birds and is associated mainly with raw poultry and contaminated water sources. It is a fragile bacterium and can be killed with oxygen, freezing, or drying. Control



measures taken for *Salmonella*, *Listeria*, and *E. coli* O157:H7 will control other pathogens such as *Campylobacter* and *Y. enterocolitica*, a pathogen associated with pork.

Staphylococcus aureus (S. aureus) (Staph)

Transmission	oral ingestion of vegetative cells
Source	meat and meat products, poultry, and egg products
Immediate symptoms	nausea, vomiting, retching, abdominal cramping, headaches, muscle cramping, and transient changes in blood pressure and pulse rate may occur
Long-term consequences	Not Applicable
Onset time	usually rapid (30 min. to 8 hrs.)
MID	greater than 100,000 cells per gram (less than 1.0 microgram of enterotoxin A)
Length of disease	generally 2 days (3 days and sometimes longer in severe cases)

In food processing, *S. aureus* is of concern in ready-to-eat food products. There are no specific regulatory requirements for controlling of *S. aureus* on RTE foods.

Staphylococcus aureus is a Gram-positive bacterium (coccus), which appears in clusters resembling grapes under the microscope. It is a non-motile, non-spore-forming facultative anaerobe that can grow with or without oxygen. It grows by aerobic respiration or by fermentation yielding lactic acid. It can grow at temperatures between 35.9-113 ˚F (7-45 ˚C), with an optimum temperature of 98.3 ˚F, (37 ˚C), a pH range of 4.2-9.3, and at salt concentrations as high as 25 percent. It is resistant to drying, and is able to produce enterotoxins in foods with a_w as low as 0.85.

S. aureus is associated with mucous membranes (nose and throat) and is commonly found on the skin and hair of healthy humans and animals. It also is

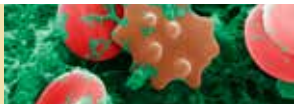
present in infected wounds, lesions, and boils in both humans and animals. It's easily spread though the air by coughing and sneezing and can contaminate meat from the animal skin or tissue during slaughter (especially during hide removal and gutting). *S. aureus* is not a good competitor with other microorganisms and, thus, usually is not a problem in raw foods. It becomes a problem when competitive microbes are removed by cooking or inhibited by high-salt levels. In salted meats, where many microorganisms are inhibited, *S. aureus* can flourish without proper controls. Nitrite does not affect the growth of *S. aureus* in air-dried fresh pork sausage. Even though *S. aureus* can grow with or without air, it grows best aerobically.

Staphylococcal food poisoning is caused by the consumption of a heat-stable enterotoxin produced as a byproduct during the growth of certain strains of *S. aureus*. Toxin production requires considerable growth by the microbe and is normally not present until the total cell numbers reach 100,000 per gram of meat. Since the microorganism is readily destroyed by heat, but the toxin is heat stable, total counts of *S. aureus* may not indicate if the toxin is present.

One of the biggest concerns of this pathogen is the increased incidence of methicillin-resistant *S. aureus* (MRSA) and other strains that are resistant to a variety of different antibiotics. Furthermore, *S. aureus* strains can exhibit resistance to antiseptics and disinfectants, including quaternary ammonium compounds.

Foods that require considerable handling during preparation and are kept at slightly elevated temperatures after preparation are frequently involved in staphylococcal food poisoning. Human intoxication is caused by ingesting enterotoxins produced in food by some strains of *S. aureus*, usually because the food has not been kept hot enough (140 °F [60 °C] or above) or cold enough (45 °F [7.2 °C] or below).

Staphylococci exist in air, dust, sewage, water, milk, and food, or on food equipment, environmental surfaces, humans, and animals. Humans and animals are the primary reservoirs. Animals and poultry carry *S. aureus* on parts of their body, which can lead to infections. The udders and teats of cows, tonsils and skin of pigs, and skin of chickens and turkeys are known sources. Staphylococci



are present in the nasal passages and throats and on the hair and skin of 50 percent or more of healthy people. Although food handlers are usually the main source of food contamination in food poisoning outbreaks, equipment and environmental surfaces also can be sources of contamination with *S. aureus*.

S. aureus is highly vulnerable to destruction by heat treatment and nearly all sanitizing agents when correctly applied. While heat processing (pasteurization) and normal cooking temperatures are effective in killing the pathogen, food establishments have to be alert that the enterotoxins are not inactivated by heat. Its heat resistance is increased in dry, high-fat and high-salt foods, and the organism survives frozen storage. The presence of this bacterium or its enterotoxins in processed foods or on food processing equipment (in areas that are difficult to clean) is generally an indication of poor sanitation and/or time and temperature abuse. Foods that present the greatest risk are those in which a heat treatment (e.g., cooking) or application of an inhibitory agent or treatment (e.g., cured, salted meats) has been applied.

Clostridium botulinum (C. botulinum) (botulism)

Transmission	oral ingestion of neurotoxin
Source	canned or fermented meat, vegetables, and fish
Immediate symptoms	dry mouth, dizziness, weakness; nausea and vomiting also may occur. Neurologic symptoms will follow, including blurred or double vision, the inability to swallow, difficulty speaking, descending weakness of skeletal muscles and, ultimately, respiratory paralysis. Untreated, this can lead to death.
Long-term consequences	Not Applicable
Onset time	12-48 hours
MID	a few nanograms of toxin
Length of disease	days to months

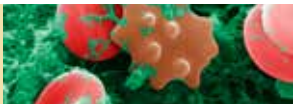
In food processing, *C. botulinum* is of concern in thermally processed ("canned") and fermented foods. There are specific regulatory requirements for controlling of *C. botulinum* in canned and fermented foods.

C. botulinum requires anaerobic conditions, so it can only grow in the absence of oxygen. Under the right conditions, it creates heat-stable spores that can survive in foods that are incorrectly or minimally processed. *C. botulinum* produces a potent neurotoxin, which is formed during vegetative cell growth. Illness is caused by the ingestion of foods containing the neurotoxin. The toxin is heat labile and can be destroyed if heated to 176 °F (80 °C) or higher for 10 minutes. Foods with a pH 4.6 or higher (more basic) can support growth and toxin production.

The ability to form spores enables *C. botulinum* to survive a wide range of unfavorable conditions, such as heat and chemicals. The spores survive many heat processes that kill other pathogens in meat and poultry. In fact, certain types of *C. botulinum* spores are able to survive 5 to 10 hours in boiling water. In canned products, oxygen is excluded from sealed containers, thus providing the anaerobic environment for spores to germinate, become vegetative cells, multiply, and produce toxin when the foods are stored at temperatures that allow growth. It is important to recognize that it is not the spore that produces the toxin, but the vegetative cell. If spores are present, but are prevented from forming vegetative cells, as in acidified foods, the toxin will not be produced.

In summary, *C. botulinum* is of great concern to home and commercial canners because:

- It can produce a potent neurotoxin when it grows.
- It can be isolated from soil or water practically everywhere in the world.
- It is the pathogen with the greatest heat resistance due to its ability to produce heat-resistant spores.
- Canning foods provides an anaerobic environment favorable to the growth of the organism if it has not been destroyed by the canning process.



Clostridium perfringens (C. perfringens)

Transmission	oral ingestion of vegetative cells
Source	cooked meat and poultry dishes, beans
Immediate symptoms	explosive diarrhea, abdominal cramps, occasionally nausea. A severe complication, necrotic enteritis, can develop if large numbers of bacteria are ingested.
Long-term consequences	Not Applicable
Onset time	8-16 hours
MID	greater than 1,000,000,000 cells (10^8)
Length of disease	1 day

In food processing, *C. perfringens* is of concern in ready-to-eat food products. There are specific regulatory requirements for the control of *C. perfringens* in RTE foods.

C. perfringens requires anaerobic or microaerophilic conditions, so it can only grow when there is little or no oxygen. It can be present in two forms, vegetative cells and spores. Illness is caused when the vegetative cells are ingested. Environmental conditions in the body cause the vegetative cells to form spores. The heat-sensitive toxin is produced in the gastrointestinal tract during the sporulation process (enterotoxin). To get sick, it is necessary to consume a large number of vegetative cells.

Intoxication is often caused by eating temperature-abused leftovers. *C. perfringens* forms spores that can withstand cooking temperatures. When the foods are cooled, spores germinate, and the germinated cells multiply. If the food is eaten without adequate reheating, *C. perfringens* enters the small intestine and synthesizes enterotoxins.

The vegetative cells are killed by heat applied during the production of RTE foods. However, the spores are heat resistant, so they may survive cooking, and the heat applied during the RTE production will cause the spores to germinate. The resulting vegetative cells will grow to large numbers during the post-cooking cooling period ("stabilization"), particularly if the cooked food is held between 40 °F and 140 °F for an extensive period.

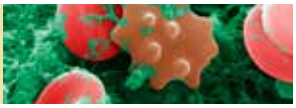
Molds

Molds exhibit some of the characteristics of the higher plants. They are multiple cell organisms forming tubular filaments. Molds demonstrate branching and reproduce by means of fruiting bodies, called spores, which are borne in or on aerial structures. Their mycelia, or intertwined filaments, may resemble roots. They are many times larger than bacteria and somewhat longer than yeasts.

Molds are widely distributed in nature, both in the soil and in the dust carried by air. Under suitable conditions of moisture, air and temperature, molds will grow on almost any food. The black or green discoloration that appears on moldy bread is familiar evidence of such growth. Molds also are able to survive on a wide variety of substances not normally thought suitable for the support of life. These include concentrated solutions of some acids and water containing minute quantities of certain salts, as well as on building structures. Molds grow readily on the walls and ceilings of buildings where there is high humidity and considerable moisture condensation. Mold growth can even occur in refrigerators, because molds are much more tolerant to cold than heat. Molds can grow at reduced water activities and can be a problem in improperly processed dry and semi-dry fermented products. Molds, such as *Aspergillus*, *Rhizopus*, and *Penicillium,* are responsible for the spoilage of cured meats. Some molds, in the right conditions, produce mycotoxins. Molds can also cause allergic reactions and respiratory problems.

Molds are capable of consuming acids, thereby raising the pH of products. On very rare occasions, their growth in foods has removed the acid conditions that inhibit the growth of *C. botulinum*; however, never in meat or poultry products.

Most molds have little heat resistance and cannot survive the thermal processes for low-acid canned foods. Some molds produce a type of spore (ascospore) that is more resistant to heat, but these spores are much less resistant than the bacterial spores that are the target of processes for low-acid and acidified canned foods. These heat-resistant molds have not caused problems in meat and poultry products. Therefore, molds are present in canned meat and poultry products only as a result of gross under-processing or as a post-processing contaminant. Since molds must have oxygen to grow, only slight growth can occur, unless the food container has an opening to the outside environment.



To date, mold growth in thermally processed, commercially sterile, and shelf-stable foods has not been shown to present a public health problem. In fact, mold is used in the ripening process of some sausages.

Although not normally a problem in meat and poultry products, *Aspergillus flavus* (*A. flavus*) and *A. parasiticus* are two molds of importance as potential foodborne pathogens. At a favorable temperature, around 77-86 °F (25-30 °C), and moisture level of 13-18 percent, certain strains of these molds produce mycotoxins known as aflatoxins, which have been found as contaminates in tree nuts, peanuts, other oilseeds (including corn and cottonseed), and milk. The major aflatoxins of concern are B1 (the most toxic), B2, G1, and G2. Aflatoxins cause aflatoxicosis, characterized by the acute death of tissue, cirrhosis, and carcinoma of the liver in a number of animal species. Although aflatoxicosis is rarely reported in humans, it is believed that the aflatoxins would have the same effects on humans as it does in animals. Aflatoxicosis may occur in both an acute and chronic form, and it may go unreported because it is not recognized as the underlying cause of a patient's illness. Treatment is supportive (monitoring of liver function, dialysis, giving intravenous fluids) and an absorbent substance, such as activated charcoal, may help. Aflatoxins are inactivated by reagents such as concentrated ammonia and sodium bisulfate.

Yeasts

Another microorganism of importance to food preservation/spoilage is yeast. Yeasts are single-cell, microscopic living bodies, usually egg-shaped. They are smaller than molds, but larger than bacteria. Their greatest thickness is about 1/2,000 of an inch. Yeasts reproduce mainly by budding. A small bud forms on the parent yeast cell, gradually enlarges, and then breaks off into another yeast cell. A few varieties reproduce by forming spores within a special cell; later, these spores may form new yeast cells.

Yeasts are widely found in nature and are particularly associated with liquid foods containing sugars and acids. They are quite adaptive to adverse conditions such as acidity and dehydration. Like molds, yeasts are more tolerant to cold than to heat. Compared to bacterial spores, yeasts and their spores possess little resistance to heat. Heating to 170 °F (77 °C) destroys most yeast forms. In canned food, the presence and growth of yeast may

result in spoilage, generally in the form of alcohol production and large amounts of carbon dioxide gas, which swells the container. If this happens, gross underprocessing, post-processing contamination, or leakage must be suspected.

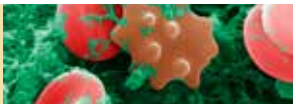
Yeast growth in processed foods does not present a public health problem.

Viruses

Virus particles are so small they cannot be seen by the standard light microscopes used in laboratories. A special electron microscope is needed to see these microorganisms. A virus particle is composed of either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) enclosed in a coat of protein, sometimes with an outer envelope containing lipids (fats). DNA is a nucleic acid which carries genetic instructions for the biological development of all cellular forms of life and many viruses. RNA transmits genetic information from DNA to proteins and carries the genetic instructions for many viruses. Viruses lack the enzymes and other components needed to replicate. Thus, viruses cannot multiply in food; they can only replicate themselves in suitable living host cells. Viruses transmitted by food are produced in the human body and shed in the feces. Of particular concern for foods are the hepatitis viruses and noroviruses. Norovirus have been mentioned in many news stories in recent years for causing large outbreaks of vomiting illnesses at large gatherings and on cruise ships. Viruses get into food through contaminated water and infected food handlers with poor hygienic practices.

Viruses are not heat resistant, with most having resistance similar to non-spore forming bacteria. Hepatitis A virus is somewhat more resistant, but is still inactivated at 185 °F (85 °C). Avian influenza virus, which can infect chickens, turkeys, pheasants, quail, ducks, geese, and guinea fowl, as well as a wide variety of other birds, has been known to infect humans, but it is not transmitted through foods. Nor is the exotic Newcastle disease virus, which also causes a highly contagious poultry disease. Heating product to an internal temperature of at least to 161.6 °F (72 °C) is considered adequate to inactivate both these viruses.

Viruses are not a concern in thermally processed, commercially sterile, and shelf-stable meat and poultry products handled under good hygienic conditions.



Parasites

The parasites of concern in the production of meat and poultry products include worms and protozoa. Some of them are large enough to see with the naked eye, whereas others are microscopic. Parasites cannot multiply in food. They can only multiply in a host cell, and are not heat resistant.

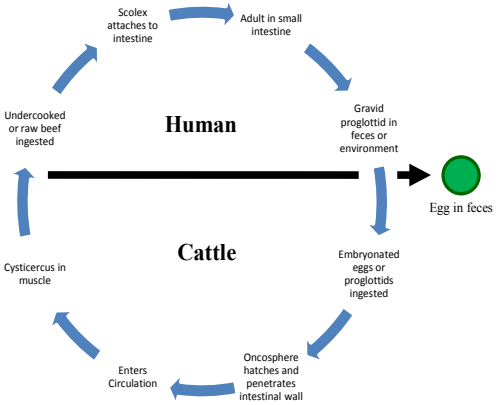
Parasitic worms of public health importance are the beef and pork tapeworms (*Taenia saginata* and *Taenia solium*, respectively) and the roundworm that causes trichinosis (*Trichinella spiralis*, also referred to as trichinae) found in pork.

The small cysticerci (immature larval stage in a cyst known as a cysticercus when there is only one and cysticerci when there is more than one) of *Taenia saginata* and *Taenia solium* are approximately 6-18 mm wide by 4-6 mm in length when found in the muscles or under the skin, the normal sites for the larvae of this parasite. The cysticerci may, however, be found in other tissues, such as those of the central nervous system, where they may grow to several centimeters in diameter.

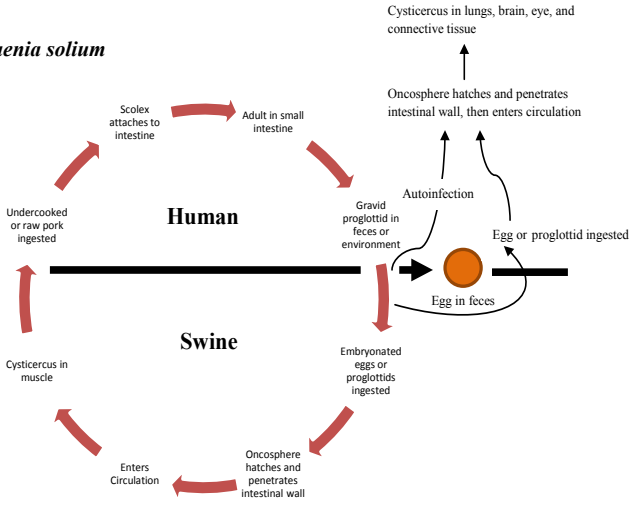
Muscle and organs of animals with severe tapeworm infection are usually visually detected by Government inspection personnel or by plant employees when there is evidence of the cysticerci of tapeworms in the muscles. These products cannot be further processed for human consumption. When the cysts are less severe or evident, infected meat may enter the human food chain and will not cause illness if the meat is properly cooked. Humans consuming undercooked meat infected with these tapeworms become ill with taeniasis generally after the mature stages of the tapeworms, which develop from the cysticercus, invade the intestinal tract. Most cases of infection with adult worms are without symptoms. Some persons may experience abdominal pain, weight loss, digestive disturbances, and possible intestinal obstruction. Taeniasis may last many years without medical treatment. However, people can get a more serious illness called cysticercosis by consuming food or water contaminated with the eggs of *Taenia solium* (pork tapeworm). The worm eggs hatch, and the larvae then migrate to various parts of the body and form cysts (cysticerci). This can be a serious or fatal disease if it involves organs, such as heart or eyes, or an organ system, such as the central nervous system. Symptoms may vary depending on the organ or organ system involved. For example, an individual with cysticercosis involving the central nervous system

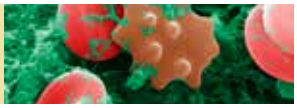
(neurocysticercosis) may exhibit neurological symptoms such as psychiatric problems or epileptic seizures. Death is common.

Life Cycle of *Taenia saginata*



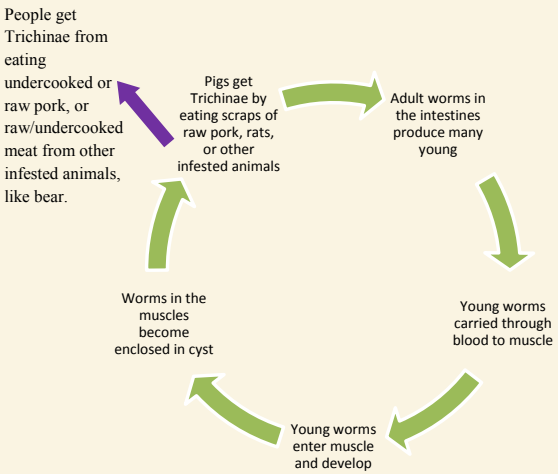
Life Cycle of *Taenia solium*





*Trichinella spiralis (*Trichinae*)* is an intestinal worm that produces larvae that migrate to and encyst in muscles of a number of animals, particularly swine. Humans consuming infested pork that is undercooked get ill from the cysts, which then live in the muscles of the human hosts. The first symptoms are nausea, diarrhea, vomiting, fever, and abdominal pain, followed by headaches, eye swelling, aching joints and muscles, weakness, and itchy skin. In severe infestations, persons may experience difficulty with coordination and have heart and breathing problems. Death may occur in severe cases.

Life Cycle of *Trichinella spiralis*



Parasitic protozoa of concern in meat processing include *Cryptosporidium parvum* and *Toxoplasma gondii*. *Cryptosporidium* is typically transmitted to humans from the fecal material of animals, primarily cattle, via contaminated water or, occasionally, food that has come in contact with contaminated water. The organism is destroyed by boiling water. *Toxoplasma gondii* is carried by cats, but can infect many warm-blooded animals. A form known as the oocyst is shed and can sporulate and survive in soil and other environments for extended times; the sporulated oocyst is infectious to all warm-blooded hosts. When ingested, the sporulated oocysts go through several forms, eventually forming cysts in tissue, such as muscles. These cysts are infective if ingested. *Toxoplasma* can cross the placenta and affect the fetus, resulting in blindness

and more serious effects in the brain. It is commonly transmitted to humans by eating undercooked meat of an infected animal or by inhaling the dried feces of cats when cleaning the litter box.

Given a cool, damp environment, these protozoa may survive in moist foods for months; however, they won't multiply in foods. Protozoa may be introducted into food by infected food handlers, from contaminated water used in the finished product stage of processing or washing processing equipment, and from ingredients contaminated on the farm. [David Dawson/International Journal of Food Microbiology 103 (2005)].

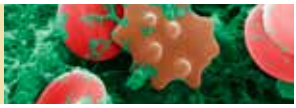
Control of Foodborne Pathogens

Primary Source of Microorganisms in Food

Raw materials and ingredients are the primary source of food contamination. Although muscle tissue is generally considered to be sterile, exposed muscle quickly becomes contaminated during the slaughter/dressing process and supports the growth of spoilage microorganisms, as well as pathogens.

The bacterial pathogens that are most likely to be found in livestock and poultry include *Salmonella, Campylobacter*, and *L. monocytogenes*. *L. monocytogenes* also is widespread in the environment. *E. coli* also are found in livestock and poultry, but most strains are not pathogenic. The pathogenic *E. coli* of primary concern is known as *E. coli* O157:H7 and is found in beef. Some strains of non-O157:H7 Shiga-toxin-producing *E. coli* (non-O157:H7 STEC) are becoming recognized as an important cause of foodborne illness. *Y. enterocolitica* is a pathogen most commonly associated with pork; only certain serotypes are pathogenic.

Some bacteria, such as *C. perfringens* and *C. botulinum,* also can be found in meat and poultry, forming spores that may survive cooking and increase in numbers in foods due to temperature abuse, though *C. botulinum* may be found in far fewer numbers than *C. perfringens*. *B. cereus* is another spore-former of concern in meat and poultry products, especially products containing dry ingredients or spices, which are a common source of the spores.

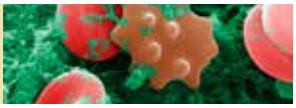


The following list outlines some of the most common ways in which microorganisms enter food products:

- **Soil, water, and in-plant environment**
  Many bacteria are carried in soil and water, which may contaminate food. These include, but are not limited to, *Listeria, Clostridium, Salmonella,* and *Escherichia.* The in-plant environment also may be an important source of contamination due to daily activities or pest infestation.
- **Animal feeds**
  This is a source of *Salmonella* for poultry and other farm animals. It is a known source of *L. monocytogenes* to dairy and meat animals when fed silage. The organisms in dry animal feed are spread throughout the animal environment and may occur on animal hides, hair, feathers, etc.
- **Animal hides**
  The hide is a source of bacterial contamination, particularly from *E. coli* O157:H7, *Salmonella,* and *Listeria*, and some spoilage organisms. The hides may contaminate the general environment, hands of plant employees, and skinned carcasses through dressing procedures during the slaughter process.
- **Gastrointestinal tract**
  The normal intestinal microflora consists of many organisms; notable among these are pathogens such as *Salmonella, Campylobacter,* and *E. coli* O157:H7. Not all of these bacteria are present in every animal species slaughtered in federally inspected establishments.
- **Establishment and agency employees**
  The microorganisms on the hands and outer garments of handlers generally reflect the environment and habits of individuals (hygiene) and may come from hides, gastrointestinal tracts, soil, water, dust, and other environmental sources. This is generally the source of viruses, primarily Norovirus and Hepatitis A.

- **Food utensils and equipment**
  Utensils, such as saws, cutting boards, knives, grinders, and mixers, may become contaminated during slaughter and processing. These surfaces may retain contaminated product when they are not properly cleaned or maintained, and, therefore, serve as a vector for cross-contamination.
- **Food ingredients**
  Food ingredients, such as spices or seasonings, may be contaminated with pathogens. The spice or seasoning can cause illness if it is added to the food product after the lethality treatment. An example of this is *Salmonella* in black pepper used to coat dried sausages.
- **Air and dust**
  A variety of bacteria may be found in air and dust in food-processing operations. *L. monocytogenes* is an example of a foodborne pathogen that survives in the environment. As air and dust move through the processing facility, they can transport pathogens.
- **Condensation**
  Water droplets that form on overhead structures because of poor air circulation can carry a variety of microorganisms. When the droplets contact food or food contact surfaces, the product may be contaminated.
- **Vegetables (plant) and vegetable products**
  Vegetables and vegetable products are a concern in the processing of meat, poultry, and processed egg products. A good example is the processing of frozen entrees, salads, etc., that contain meat and poultry components. Dried herbs and spices can be a primary source of bacterial spores since the spores will survive for extended times in the dehydrated product. Soy and milk protein ingredients also can be sources of spores.

As you can tell from reading this, microorganisms can enter food products through many different routes because they are found virtually everywhere.



Conditions Affecting Microbial Growth

Like all other living organisms, bacteria need a certain environment to live and grow. Broadly, the bacteria involved in food processing need very similar environmental conditions, although there are some important variations between bacterial species.

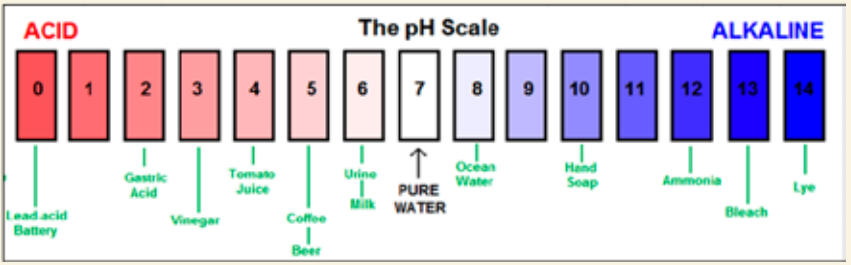
There are six basic environmental conditions needed for bacterial growth. One easy way to remember these conditions is to use the easy-to-remember acronym FAT TOM: **F**ood, **A**cidity, **T**ime, **T**emperature, **O**xygen, and **M**oisture.

- **F**ood (nutrient requirements)

  A suitable food supply is the most important condition affecting the growth of bacteria. Every living cell requires certain nutrients to multiply. These include solutions of sugars or other carbohydrates, proteins, and small amounts of other materials such as phosphates, chlorides, and calcium. If the food supply is removed, bacteria will not multiply.

- **A**cidity (pH Requirements)

  The term pH refers to the acidity or alkalinity of an aqueous solution. The pH scale ranges from 0 to 14, with pH 7 being neutral. Numbers smaller than 7 indicate a more acidic condition; numbers greater than 7 indicate a more basic, or alkaline, condition. All bacteria have an optimum pH range for growth — generally around neutral pH — as well as a minimum and maximum. The pH of foods can be adjusted to help control bacterial growth.



pH Ranges of Some Common Foods	
Food	pH Range
Beef (ground)	5.1–6.2
Ham	5.9–6.1
Veal	6.0
Chicken	6.2–6.4
Fish (most species)	6.6–6.8
Corn (sweet)	7.3
Egg yolks (white)	6.0–6.3 (7.6–9.5)
Olives (green)	3.6–3.8

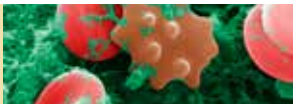
- **Time**

  Time is a factor in pretty much all of the other five environmental conditions. The more amount of time bacteria spend in favorable conditions, the higher the rate of reproduction. Remember, the amount of time spent in the lag phase of bacterial growth determines how quickly a pathogenic bacteria reaches the MID. The length of the phase affects the effectiveness of any interventions that your plant has in place to control the numbers of pathogenic bacteria.

- **Temperature**

  As with pH, all bacteria have an optimum temperature range for growth and a minimum and maximum temperature below or above which they cannot grow. Bacterial groups bear names that indicate their relationships to temperature – psychrophile, psychrotroph, mesophile, and thermophile.

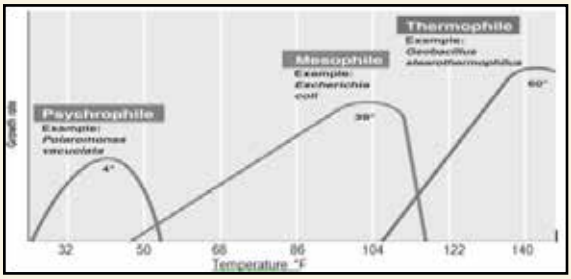
  - Psychrophilic ("cold loving") and Psychrotrophic group.
    - Both grow above the temperature range of subzero to 68 °F (20 °C).
    - Psychrophiles have an optimum temperature of 59 °F (15 °C) and cannot grow above 77 °F (25 °C).
    - Psychrotrophs grow best between 77 °F and 104 °F (25 °C to 40 °C), but can grow slowly in or on food at refrigerator temperatures (around 40 °F [4 °C]).



- Both are primarily responsible for the spoilage of refrigerated foods.

- Mesophilic group (meso = middle).
  - Grows best at temperatures of 86 °F to 104 °F (30 °C to 40 °C).
  - Some grow well at higher temperatures, such as 116 °F (46.7 °C).
  - All of the bacteria that affect food safety grow within this mesophilic temperature range, although some may be considered psychrotrophic as well.
- Thermophilic group ("heat loving").
  - Grow at high temperatures.
  - Found in soil, manure, compost piles, and even hot springs.
  - Not pathogenic and do not produce toxins during spoilage of foods; therefore, they do not affect food safety.

The graph below shows the temperature growth ranges:

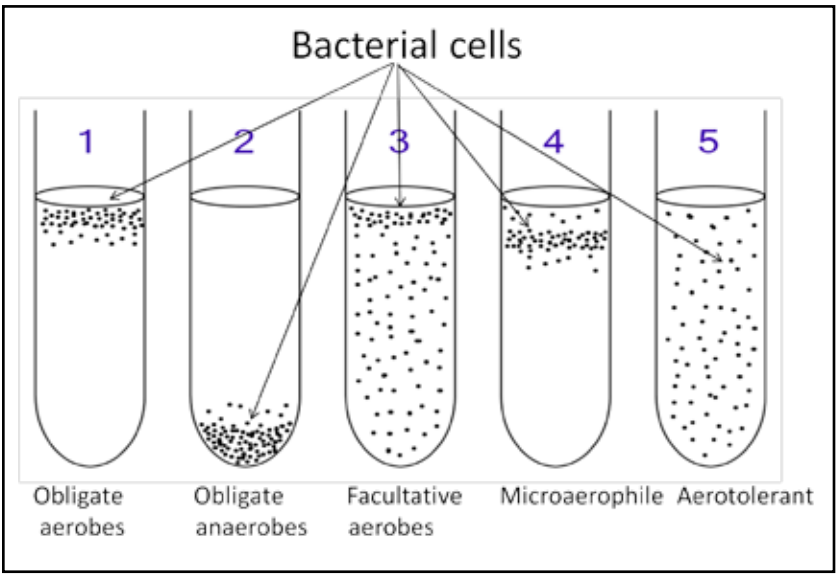


- Oxygen.

  Bacteria can be placed into groups based on their need for oxygen.
  - Aerobes require free oxygen to survive.
    - Obligate aerobes need a high concentration of oxygen.
    - Microaerophiles need a lower concentration of oxygen than obligate aerobes.
  - Anaerobes cannot grow if free oxygen is present.

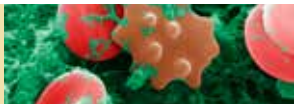
- Obligate anaerobes must avoid all oxygen.
- Facultative anaerobes use oxygen when it is present but do not need it to grow. The majority of bacteria are facultative anaerobes.

- Aerotolerant bacteria are not affected by oxygen.



- Moisture.

Nutrients must enter the bacterial cell through its cell wall, therefore nutrients must be soluble so they can be carried into the cell by any free (or unbound) water that is available in the environment where the bacteria are living (such as in food). The measure of this available free water is known as the water activity, or a_w. The a_w is the ratio of the vapor pressure of a substance (p) to the vapor pressure of pure distilled water (p_o): $a_w = p/p_o$. If you multiply the a_w by 100, you get the equilibrium relative humidity expressed as a percent (so dividing the equilibrium relative humidity by 100 gives you the a_w). The a_w is a fraction between 0 and 1.00 (1.00 is the a_w of pure water). Most bacteria need an a_w greater than or equal to 0.95 for growth.



Water Activity of Some Common Foods	
Food	**a_w**
Perishable and canned foods (including meats, vegetables, fish and milk)	0.95-1.00
Liverwurst	0.96
Some cheese spreads	0.95
Some cheeses and cured meats	0.91-0.95
Chorizos	0.92
Many fermented sausages	0.87-0.91
Semi-moist pet food	0.83
Salami	0.82
Chocolate syrups	0.75-0.83
Jams	0.75-0.80
Peanut butter – 15% total moisture	0.70
Jerky	≤0.80
Chicharones	0.32-0.40

Control of Bacterial Growth

The term "exponential growth" is intended to convey the idea that the log-converted numbers of bacteria increase in a linear fashion over a unit of time. The relation of log CFU (colony-forming units) to time is a constant value (k). Just as bacterial cells reproduce logarithmically (exponentially), they also die logarithmically. Log reduction is a mathematical term used to show the relative number of pathogenic microorganisms that are killed or inactivated by a particular process or combination of processes, such as sanitizing, disinfecting, or cleaning. Thermal reductions in bacterial number are expressed as the relation of log CFU to time held at a given temperature. Again, the relationship should be linear. The D value is the time (at a given temperature) resulting in a 1 log reduction in CFU.

This is what log reductions look like:

Log Reduction	Number of Bacteria
1	10 times fewer
2	100 times fewer
3	1,000 times fewer
4	10,000 times fewer
5	100,000 times fewer
6	1,000,000 times fewer
7	10,000,000 times fewer

As a plant owner or operator, this is an important concept to understand. Bacterial log reduction is required by certain regulatory performance standards, specifically for *Salmonella* and *E. coli* O157:H7. It also can be part of a pathogen modeling program report generated as a response to a critical limit deviation from a critical control point in your Hazard Analysis and Critical Control Point (HACCP) plan.

To achieve the necessary log reduction of pathogenic bacteria, their growth must be controlled. This control may be achieved through manipulation of the bacterial environment, the use of certain chemicals, or an interaction of these factors.

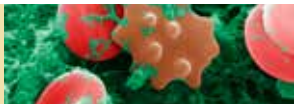
First, let's look at ways you can control bacteria through the manipulation of their environment.

- Control by pH
  - Bacterial growth can be controlled by reducing the pH below the minimum level for growth of the organism. For example, acidification can be used to prevent the growth of *C. botulinum*. However, because some bacteria, such as *E. coli* O157:H7, have low infectious doses (only a few cells are needed to cause disease), preventing growth alone will not provide a safe product. Reduced pH can be combined with other factors to control the pathogens of concern. For example, a reduced pH, combined with a mild heat treatment, is used to achieve commercial sterility in canned, acidified meat and poultry products, such as pasta sauces containing meat.
  - Acidification procedures

    To produce products with a pH of 4.6 or less, acidification must be properly done.

    - Direct batch acidification-Ingredients are mixed in a kettle with an acid or, more commonly, an acid food is added directly to the batch.
    - Addition of acidified brine, such as with pickled pigs' feet.
  - Determination of pH

    The most important factor in the production of acidified foods is the timely attainment and maintenance of a pH level that will

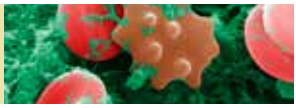


inhibit the growth of *C. botulinum* spores. To achieve this goal, it is necessary to measure pH. Although pH can be measured using dye solutions and pH paper, the recommended method for determining pH is with a pH meter. **FSIS currently requires a pH meter to be used any time pH is specified as a critical factor for a scheduled process for thermally processed product**.

Approximate pH Requirements for Microorganism Growth			
Microorganism	Minimum	Optimum	Maximum
Clostridium perfringens	5.5–5.8	7.2	8.0–9.0
Campylobacter spp.	4.9	6.5–7.5	9.0
Clostridium botulinum toxin	4.6		8.5
Clostridium botulinum growth	4.6		8.5
Staphylococcus aureus growth	4.0	6.0–7.0	10.0
Staphylococcus aureus toxin	4.5	7.0–8.0	9.6
Enterohemorrhagic *Escherichia coli*	4.4	6.0–7.0	9.0
Listeria monocytogenes	4.39	7.0	9.4
Salmonella spp.	4.2	7.0–7.5	9.5
pH minimum as low as 3.8 has been reported for *Salmonella* spp.			

- Control by temperature
  - Bacterial growth can be controlled by keeping food at temperatures above or below the temperatures at which bacteria grow. Thus, refrigeration, freezing, and hot holding can be used to control growth. However, the most effective way to control microorganisms is to kill them with heat. The amount of heat needed depends on a number of factors, including:
    - The specific microorganism (the species and whether or not it is in the spore form);
    - The number of microorganisms to be inactivated;
    - The food product; and
    - Factors of the food, such as its pH, a_w, the presence of preservatives (such as nitrite), and the amount of fat.
  - The Danger Zone

    The "danger zone" is a term used to describe the temperature range that provides bacteria the optimal growth conditions. This range is 40 °F to 140 °F (4 °C to 60 °C).
    - Design food processes to ensure that food products spend as little time as possible in the danger zone.
    - Control the entire danger zone temperature. At the high end, bacteria will grow slowly, and at the low end, many bacteria will survive and some may grow.
    - Temperatures above the danger zone begin to destroy most microbes, although the time needed for cell destruction is longer at lower temperatures. At refrigeration temperatures, some spoilage bacteria and a few pathogens can grow very slowly; however, for most bacteria, refrigeration temperatures are too cold for optimal growth. No significant bacterial growth occurs below freezing.



Approximate Temperature Requirements for Microorganism Growth in °F (°C)			
Microorganism	Minimum	Optimum	Maximum
Campylobacter spp.	90 (32)	108–113 (42–45)	113 (45)
Clostridium botulinum types A & B	50–54 (10–12)	86–104 (30–40)	122 (50)
Clostridium botulinum type E	37–38 (3–3.3)	77–99 (25–37)	113 (45)
Clostridium perfringens	54 (12)	109–117 (43–47)	122 (50)
Enterohemorrhagic Escherichia coli	45 (7)	95–104 (35–40)	115 (46)
Listeria monocytogenes	32 (0)	86–99 (30 –37)	113 (45)
Salmonella spp.	41 (5)	95–99 (35–37)	113–117 (45–47)
Staphylococcus aureus growth	45 (7)	95–104 (35–40)	118 (48)
Staphylococcus aureus toxin	50 (10)	104–113 (40–45)	115 (46)

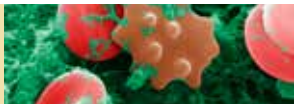
- Control by a_w
  - When substances are dissolved, there is a substantial reaction between the substance and the water. The water-binding capacity of a particular dissolved ingredient influences the amount of water left for the growth of bacteria.
    - A number of the molecules of the water are bound by the molecules of the substances dissolved.
    - All of the substances dissolved in the water reduce the number of unattached water molecules and, in this way, reduce the amount of water available for bacterial growth.
    - The extent to which the water activity is lowered depends primarily on the total concentration of all dissolved substances. The dissolved substances compete with the bacteria for available water.

- The a_w is a fraction between 0 and 1.00, with the a_w of pure water being 1.00. The measurement of food's a_w provides information as to which types of microorganisms are most likely to cause spoilage and how close the a_w is to the safety limit.
  - Most bacteria, yeasts, and molds will grow above a water activity of 0.95, and most foods have a water activity above 0.95.
  - Spores of *C. botulinum* are generally inhibited at an a_w of about 0.93 or less. Thus, one food preservation method is to reduce the amount of water available to spores to a point where they are inhibited and apply a mild heat treatment to destroy the vegetative cells. Examples of meat products preserved with mild heat and reduced a_w are jerky and some dry sausages.
- Methods for determining a_w
  Several methods exist for determining the a_w of a food.
  - One commonly used method is an electric hygrometer with a sensor to measure equilibrium relative humidity (ERH). The sensors are the same as those used to measure relative humidity in air.
  - A dew point instrument is also commonly used to measure a_w.

Some Minimum a_w Requirements for Microorganism Growth

Microorganism	Minimum a_w for Growth
Most molds (e.g., *Aspergillus*)	0.75
Most yeasts	0.88
C. botulinum	0.93
S. aureus	0.85
Salmonella	0.94
L. monocytogenes	0.92

Next, let's take a look at how you can control bacteria through the use of chemicals. Chemicals may be added to foods to inhibit bacterial growth or to kill microorganisms. However, at normal levels of use (which must be approved by regulatory agencies, such as USDA's Food Safety and Inspection Service and the Food and Drug Administration), most chemicals cause inhibition rather than inactivation. Acids and their salts (e.g., lactic acid and sodium lactate), nitrites, some phosphates, and sodium chloride (salt) are



common chemicals added to meat and poultry. Chemicals are often used in combination with other factors, such as heat or reduced a_w.

- Salt
  - Salt (NaCl) is an ingredient which lowers the a_w of food.
  - It is often supplemented with other ingredients, such as nitrites, that aid in spoilage prevention.
  - If other conditions are not optimum for growth (e.g., low pH or temperature), then less salt is required to inhibit growth. For example, growth of *C. botulinum* may occur at a water activity of 0.96 (6.5 percent NaCl) at pH 7.0, but if the pH is reduced to 5.3, growth will be inhibited at a water activity of 0.97 (5 percent NaCl). The salt content of a meat product is not as important in inhibiting bacteria as the brine concentration (percent of salt in the aqueous portion of the meat).
- Nitrite
  - One important function of nitrite in meat products is that it inhibits *C. botulinum* growth and toxin production.
  - In spite of large amounts of research, there still is not a complete understanding of how nitrite controls *C. botulinum* in meat products.
  - Nevertheless, it is now recognized that nitrite inhibition is due to a combination of factors, not nitrite alone.

Finally, you can control bacteria by utilizing the interaction of various factors that were just reviewed. Combinations of inhibitory factors that alone may be insufficient to control microorganisms can often be effective when used together. This is referred to as the "hurdles," or "multi-hurdle" concept—if enough hurdles or barriers are included, bacteria will not be able to overcome the hurdles and grow. For example, when the water activity is lower, the pH range at which an organism can grow is more limited.

Food Preservation Techniques Based on Control Factors

You've probably already figured out that most food preservation techniques are based on the control factors mentioned in the previous section. The basic principle of all forms of food preservation is to control the growth of bacteria,

typically spoilage bacteria. Proper processing of food helps ensure that the growth of harmful microorganisms is controlled or eliminated. Popular techniques include:

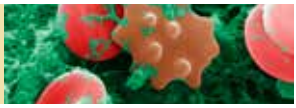
- Temperature control/refrigeration and freezing
- Canning/thermal processing
- Irradiation
- Chemical preservation
- Pasteurizing (heat)
- High-pressure pasteurization (HPP)
- Acidification/pickling and curing
- Salting
- Dehydration
- Fermentation
- Carbonation
- Freeze-drying
- Modified Atmosphere Processing (MAP)

Conditions Affecting Molds and Control of Mold Growth

Remember, molds are widely distributed in nature, both in the soil and in the dust carried by air. The only reason they might be present in canned meat and poultry products is because of under-processing or contamination after processing.

Molds can grow over a pH range of 1.5 to 9.0 and a minimum a_w 0.75. Spores can tolerate harsh environmental conditions, but most are sensitive to heat treatment. Different mold species have different optimal growth temperatures, with some able to grow in refrigerators. The optimum temperature range is 77 °F to 88 °F (20 °C to 30 °C), but some growth can occur between 32 °F and 95 °F (0 °C to 35 °C). Molds require oxygen to grow.

Since molds and their spores are sensitive to heat, they cannot survive the thermal processes for low-acid canned foods. Mold spores are not as resistant to heat as the bacterial spores that are the target of the processes used for the



production of low-acid and acidified canned foods. Since molds must have oxygen to grow, only slight growth can occur, unless the food container has an opening to the outside environment.

Conditions Affecting Yeast and Control of Yeasts

Yeasts are widely found in nature and are usually associated with liquid foods containing sugars and acids. Yeast growth in processed foods does not present a public health problem.

Yeasts are able to adapt to conditions such as acidity and dehydration that would be too adverse for other types of organisms. Most yeasts require a_w 0.87. An a_w of 0.85 or less suppresses the growth of organisms of public health significance. Like molds, yeasts are more tolerant to cold than heat. Compared to bacterial spores, yeasts and their spores possess little resistance to heat, and heating to 170 °F (77 °C) destroys most forms of yeast. Yeasts, unlike molds, are facultative anaerobes.

In canned food, the presence and growth of yeast may result in spoilage, generally in the form of alcohol production and large amounts of carbon dioxide gas, which swells the container. It this happens, gross under-processing, post-processing contamination, or leakage must be suspected.

Conditions Affecting Viruses and Control of Viruses

Since viruses get into food through contaminated water and infected food handlers with poor hygienic practices, the best preventative measures involve ensuring that only potable water from a trusted (and tested) source is used in your processing facility and reinforcing good hygienic practices among your employees. You should also not allow employees to work when ill, especially if they have symptoms such as diarrhea, nausea, and vomiting. Both Norovirus and Hepatitis A have low infectious doses—approximately 10 to 100 viral particles for Norovirus, and 10 or fewer virus particles for Hepatitis A.

Norovirus in food is not inactivated by processes used for preservation and storage, such as freezing, acidification, and moderate heat treatments (pasteurization). However, it can be effectively inactivated with heat treatments used for food preparations, such as baking, cooking, and roasting.

For the most part, Norovirus retains infectivity at 140 °F (60 °C) for 30 minutes (Green, 2007). Pasteurization is not sufficient to eliminate the virus.

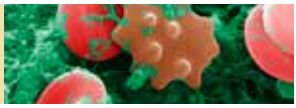
Food containing Norovirus must be heated to at least 158 °F (70 °C). Under refrigeration and freezing conditions, the virus remains intact and viable for several years. It resists gastric acids at pH 3-4. The virus retains infectivity after exposure to pH 2.7 for 3 hours at room temperature. It is believed to be sensitive to pH > 9.0, but that has not yet been proven. Based on data for other enteric viruses and virus indicators, it is likely that Norovirus persist in waters for extended periods (possibly weeks/months) (Carter, 2005; Rzezutka and Cook 2004). Norovirus has caused many waterborne outbreaks and is often detected in environmental waters. Norovirus is also resistant to drying. Infectious Norovirus has been detected on environmental surfaces, including carpets, for up to 12 days after outbreaks (Carter, 2005; Greening and Wolf, 2010). [First published November 2009, doi: 10.1128/AEM.01797-09 Appl. Environ. Microbiol. January 2010 vol. 76 no. 2 536-545]

The Hepatitis A virus is very stable; shows high resistance to chemical and physical agents such as heat, acid, and solvents; and has been shown to survive in the environment for over 3 months.

Hepatitis A integrity and infectivity were retained after incubating laboratory-prepared solutions containing the virus for 60 minutes at 140 °F (60 °C), and infectivity was retained after 10 minutes at 176 °F (80 °C). The Hepatitis A virus is even more heat resistant when present in foods and shellfish. Food containing the Hepatitis A virus must be heated to 185 °F (85 °C) or above. Under refrigeration and freezing conditions, the virus remains intact and infectious for several years. The virus is stable in acidic conditions. It retained high infectivity after 2 hours and was still infectious after 5 hours at pH 1.0 and 77 °F (25 °C). The virus is also resistant to drying. It remained infectious for over 1 month at 77 °F (25 °C) and a humidity of 42 percent. There is greater resistance to inactivation at low humidity and temperatures.

Conditions Affecting Parasites and Control of Parasites

Parasites are readily destroyed by cooking. They are not a major concern in thermally processed, commercially sterile meat and poultry products since they are subjected to temperatures well in excess of what is needed to destroy parasites. Parasites are a concern in shelf-stable products that are not cooked. For example, trichinae are a concern in shelf-stable products containing pork, such as dried sausages.



FSIS has regulations regarding the disposition of carcasses affected with *Taenia saginata* and *Taenia solium*: Title 9 of the *Code of Federal Regulations*, Section 311.23 (9 CFR 311.23), "Tapeworm cysts (cysticercus bovis) in cattle," and 9 CFR 311.24, "Hogs affected with tapeworm cysts." According to 9 CFR 311.23, if the cattle carcass shows signs of severe infestation (meaning that the lesions are "extensive" or "if the musculature is edematous or discolored"), the carcass must be condemned. Carcasses without extensive infestation may be passed for human food only after:

- the removal and condemnation of the lesions and surrounding tissues (done after careful postmortem examination),
- the carcasses are held in cold storage at a temperture of 15 °F or below continuously for a period of 10 days or more, or
- the carcasses are heated throughout to a temperature of 140 °F or more.

In the case of boned meat derived from carcasses without extensive infestation, the meat, when in boxes, tierces, or other containers, must be treated by:

- holding them at a temperature of 15 °F or below continuously for a period of 20 days or more, or
- heating the meat throughout to a temperature of 140 °F or more.

The carcasses and meat are under retention and the control of FSIS inspection program personnel until treatment is complete. Edible viscera and offal from the carcass are disposed of in the same manner as the carcass unless any lesion of cysticercus bovis is found in the byproducts. If so, they are condemned.

For tapeworm cysts in swine, 9 CFR 311.24, "Hogs affected with tapeworm cysts," states "Carcasses of hogs affected with tapeworm cysts (Cysticercus cellulosae) may be passed for cooking, unless the infestation is excessive, in which case the carcass shall be condemned." The carcasses or meat should be heated to 140 °F or more.

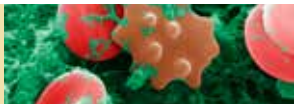
FSIS also has regulations regarding the treatment of pork and pork products for *Trichinella spiralis* – 9 CFR 318.10, "Prescribed treatment of pork and products containing pork to destroy trichinae." The regulation outlines the requirements for heating, refrigerating, and curing of pork products to kill trichinae. Essentially, all forms of fresh pork (including fresh unsmoked sausage containing pork muscle tissue and pork, such as bacon and jowls [except as

described in paragraph (b) of the regulation]), are considered products that are usually well cooked before they are consumed, so they do not have to be treated. In addition, pork from carcasses or carcass parts that have been found free of trichinae, as described under paragraph (e) or (f) of the regulation, is not required to be treated for the destruction of trichinae.

All of the products listed in paragraph (b) of the regulation, as well as products having the same character as products listed in paragraph (b), must be treated to kill trichinae. For detailed information, please review 9 CFR 318.10.

Time/temperature table for heating pork

Minimum Internal Temperature		Minimum Time
Degrees Fahrenheit	Degrees Centigrade	
120	49.0	21 hours
122	50.0	9.5 hours
124	51.1	4.5 hours
126	52.2	2 hours
128	53.4	1 hour
130	54.5	30 minutes
132	55.6	15 minutes
134	56.7	6 minutes
136	57.8	3 minutes
138	58.9	2 minutes
140	60.0	1 minute
142	61.1	1 minute
144	62.2	instant



Time/temperature table for freezing pork

Temperature °F	Group 1 (Days)	Group 2 (Days)
5	20	30
-10	10	20
-20	6	12

Group 1 comprises product in separate pieces (not exceeding 6 inches in thickness), or arranged on separate racks (with the layers not exceeding 6 inches in depth), or stored in crates or boxes (not exceeding 6 inches in depth), or stored as solidly frozen blocks (not exceeding 6 inches in thickness). Group 2 comprises product in pieces, layers, or within containers, (the thickness of which exceeds 6 inches but not 27 inches), and product in containers including tierces, barrels, kegs, and cartons (having a thickness not exceeding 27 inches).

Alternative time/temperature table for commercial freeze-drying or controlled freezing

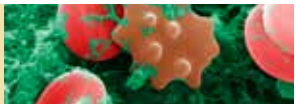
Minimum Internal Temperature		Minimum Time
Degrees Fahrenheit	Degrees Centigrade	
0	-17.8	106 hours
-5	-20.6	82 hours
-10	-23.3	63 hours
-15	-26.1	48 hours
-20	-28.9	35 hours
-25	-31.7	22 hours
-30	-34.5	8 hours
-35	-37.2	1/2 hour

The parasitic protozoa *Cryptosporidium parvum* can be destroyed by heating water to a rolling boil (212 °F or 100 °C) for at least 1 minute. *Cryptosporidium parvum* is resistant to inactivation by chlorine or iodine. Some of the factors affecting the survival of *Cryptosporidium* are [David Dawson/International Journal of Food Microbiology 103 (2005):

- **Environment** – The oocysts are resistant to cool, damp conditions. Oocysts have survived for 1 year in sea water (Tamburrini and Pozio, 1999) and 6 months in river water and cow feces (Robertson et al., 1992).
- **Heat** – The oocysts are not infective when water or milk is heated to 161.1 °F (71.7 °C) for 5, 10, and 15 seconds (Harp et al., 1996); or, when water is heated to 147.6 °F (64.2 °C) for 2 minutes (Fayer, 1994).
- **pH** – The oocysts lose viability in orange juice, carbonated beer, or cola at a pH 3.9 and a temperature of either 39.2 °F (4 °C) or 71.6 °F (22 °C), (Friedman et al., 1997). Infectivity is reduced by malic acid, citric acid, tartaric acid (Kniel et al., 2003), ethanol, and low water activity (Dawson et al., 2004).
- **Freezing** – The oocysts are not infective after freezing at -94 °F (-70 °C). However, some oocysts remain viable after 7 days at 5 °F (-15 °C) and -4 °F (-20 °C) (Fayer and Nerad, 1996).
- **Disinfectant** – The oocysts are very resistant to disinfectants. Although routine chlorination of water is not effective, the use of ozone disinfection is highly effective (Casemore, 1995).
- **Drying** – The oocysts are very sensitive to drying. In one study, 95 percent of oocysts died within 4 hours at room temperature (Robertson et al., 1992).
- **UV Light** – The oocysts are sensitive to UV light used in water treatment (Hargy et al., 2000).

The cysts of *Toxoplasma gondii*, the other parasitic protozoa, can be destroyed through heating product to an internal temperature of about 153 °F (67 °C) and freezing to a temperature of 10.4 °F (-12 °C). More specifically, the internal temperature of the product must be heated to 131 °F (55 °C) or higher and held at that temperature for at least 20 minutes, 141.8 °F (61 °C) for 3.6 minutes, or 152.8 °F (67 °C) for 7 seconds to inactivate *Toxoplasma gondii* tissue cysts.

Insect control is very important for the control of both protozoa because insects may actually transfer oocysts to uncovered food.



Microbiological Sampling Programs

Microbiological sampling is not a magic bullet. Sampling by itself will not render an adulterated or contaminated product safe. Rather, it is the antimicrobial interventions that reduce or prevent contamination or decontaminate the product. However, sampling does provide some assurance (evidenced by negative test results) that products are not adulterated, which is why it is used as a "verification" procedure. Therefore, sampling is a means of verifying that the antimicrobial interventions are working in controlling the hazard. Federally inspected establishments are not required to conduct their own microbiological sampling of their meat or poultry products, but many do for various reasons (e.g., verifying effectiveness of antimicrobial interventions, customer request, etc.).

As a small plant owner and operator, it's important for you to be aware of the fact that pathogens in food products are usually present at very low levels, if at all, and are unevenly distributed. Also, bacteria are not always predictable. Keep in mind that the food environment is complex. Some ingredients of foods will actually provide a protective environment for bacteria (e.g., fat), while other food ingredients are injurious to bacteria (e.g., acid, preservatives, etc.).

Design of a Sampling Plan

When designing a sampling plan, you should seek what you hope you won't find. Anyone can design a plan that, when followed, will result in no or negative findings. However, it is in your best interest to find the pathogens before the contaminated product enters commerce. Otherwise, the impact on public health and the associated costs to your business could be devastating. Therefore, detecting the pathogen is the desirable outcome of a well-designed and executed sampling program.

The amount and frequency of sampling depends on the amount of risk inherent to the product and on a number of variables. These include, but are not limited to, the number of antimicrobial interventions, the number of suppliers, whether you follow best practices, and how product lots are determined. Another variable that will impact risk is the level of contamination. Products with low levels of contamination must be sampled more frequently than heavily contaminated products to obtain the same level of confidence from the results.

Essential Components of a Sampling Plan

To be effective, your sampling plans need to include certain components. Those components should be clearly identified and include:

- Purpose of Sampling – Why is sampling being done?

  There are many reasons to sample product. The purpose of the bacterial sampling will impact what product is to be sampled and how. For example, is the sampling being conducted to determine if product is adulterated? Is it to determine if the food contact surfaces or product are contaminated? Is it to determine the level of contamination, as when establishing a baseline (e.g., *Salmonella* baseline study)? Is the sampling being conducted to determine the effectiveness of a procedure (e.g., sanitary dressing), or is it done to satisfy a customer requirement?

- Product To Be Sampled – What is to be sampled?

  It's most effective to sample both source materials as well as finished product. Not all source materials carry the same risk of contamination. For example, beef from advanced meat recovery systems (AMR) is more likely to be contaminated with *E. coli* O157:H7 than beef heart meat. Likewise, beef from bench trim is more likely to be contaminated with *E. coli* O157:H7 than beef manufacturing trim.

  Remember, a well-designed sampling plan is one that is designed to find the pathogen, if it is present. Since contamination is usually more prevalent on the surface of the carcass, it's logical to collect samples from the surface as opposed to collecting core samples, which would decrease the likelihood of finding the pathogen.

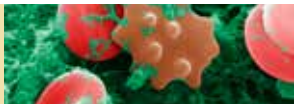
- Frequency of Sampling – When will the sampling occur?

  Since pathogens, such as *E. coli* O157:H7, are not evenly distributed throughout the lot, the portions should be collected randomly, at different sites throughout the lot, and/or at different times throughout production. FSIS' N-60 sampling is based on this type of sampling design.

- Sample Size – How much will be sampled?

  An effective sample is one that fully represents the lot.

- Analytical Method – Which test will be used?



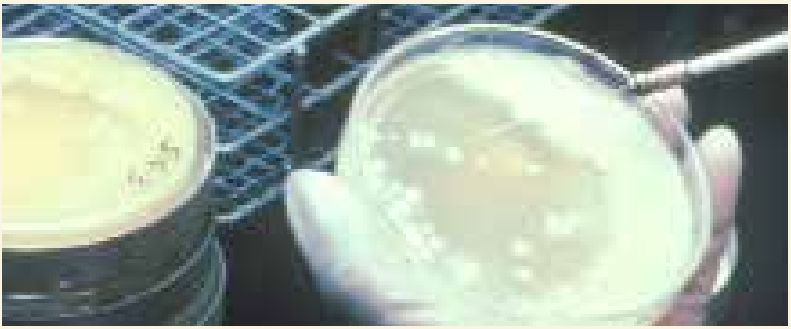
There are many microbiological sampling methods. The method used should be appropriate for the purpose. The sampling method used should be one capable of detecting the lowest possible levels of pathogens, specifically in meat or poultry products.

- Effectiveness of Analytical Method – Will the test find the target organism?

  The detection method should be able to identify intact pathogens, as well as pathogen cells that may have been damaged by antimicrobial treatments or preservation methods, such as refrigeration or freezing. Not all sampling methods used by all laboratories are capable of achieving this level of detection.

Samples should be properly and completely identified. Be sure to minimize opportunities for spoilage while transporting samples to the laboratory because growth of competing spoilage bacteria could mask the presence of pathogens in the sample and make them harder to detect. Avoid freezing samples during storage and transit because pathogens can die off during freeze/thaw cycles.

Remember that the best sampling plans provide the opportunity for detection. However, there is no guarantee of detection.



C. botulinum in a petri dish.

Product Versus Environmental and Food-Contact Surface Sampling

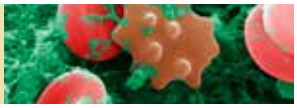
Environmental testing refers to testing of the surrounding environment in your plant, not to food contact surfaces. Food contact surface testing refers to the testing of all surfaces, including tables, belts, equipment, etc., that come in contact with your product. Environmental and food-contact surface testing assesses the control of the in-plant environment, not whether a specific lot of product has been contaminated. You use environmental and food-contact surface testing to verify the effectiveness of your sanitation procedures. Although a statistically designed program can be used, environmental and food-contact surface testing programs are often not statistically designed. Instead, they are based on prior experience and familiarity with your processes and equipment.

Environmental and food-contact surface testing is a better and more cost-effective method than product testing for assessing process control. It is used to detect trends that indicate a potential loss of sanitation control in the environment and enable timely corrective actions. Testing that focuses on controlling sanitation in the food processing environment and exposure to the risks associated with food-contact surface contamination is actually more informative than end-product sampling. For example, unlike food-contact surface sampling, end-product samples found to be positive do not indicate how the products became contaminated or how to prevent recurrences.

Niches are often involved (e.g., hollow rollers or conveyors) when a foodborne pathogen, such as *L. monocytogenes,* gets established in a RTE food processing facility. A niche is a site within the food processing establishment where bacteria become entrenched and multiply. Niches are often reservoirs where the pathogens are spread during processing and, subsequently, contaminate your product and product-contact surfaces.

Construction in areas where RTE products are exposed can increase the risk of product contamination with *L. monocytogenes*. This may be due to dust being dispersed throughout the area and can lead to the introduction of new, more virulent strains of *L. monocytogenes* into the environment.

If a construction event increased the rate of product, food contact surface, or environmental contamination, the routine sampling plan would become more effective as verification. Higher rates are more likely to be detected as positive results. It would be prudent to enhance sampling because of the overall probability of a false negative.



Under these conditions, you would need a plan to segregate the production areas from the areas under construction and to continue to verify the effectiveness of the sanitation/*L. monocytogenes* control program with testing.

Environmental and Food-Contact Surface Sampling Programs

The design of your program and your response to positive findings determines the effectiveness of the program. Routine environmental sampling and food-contact surface sampling programs are essential for maintaining an assessment of control. Positive findings should be addressed with corrective actions necessary to identify and control the source of contamination. Ideally, samples should be analyzed individually. However, compositing of samples may be done to reduce costs associated with sampling.

Sample sites should include areas found to have been good indicators of control, for example, equipment surfaces that are exposed to food, brines for chilling, or floors near packaging machines.

Sampling Frequency

A good sampling frequency is to sample from each processing line at least once a week. The number of samples should be enough to assess control (e.g., 2-10 samples). The specific number should reflect your plant's history of control and the complexity of the system. The sampling program should identify appropriate sample sites.

Analyzing Results

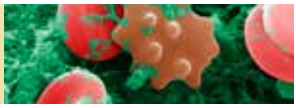
You should review results as soon as they become available. It's very important to keep in mind that positive results will occur. We know that *L. monocytogenes* is in the food-processing environment, but it's often difficult to find its specific source or location. Even with all this sampling, there is often difficulty in detecting the source. Ideally, sampling programs will reveal the extent of a problem so that your resources should be directed where risk is highest.

Positives from an environmental testing program will help you identify where the contamination is occurring in the process. Once identified, the problem can be corrected. The findings should be viewed as a success because they indicate your monitoring program has been effective in finding and identifying the pathogen.

Aseptic Sampling Techniques

An aseptic technique implies that you do not add any organisms to the sample when it is collected. It does not imply that the sample is aseptic or free of microorganisms. Extraneous microorganisms from the environment, hands, clothing, sample containers, and sampling devices may lead to erroneous analytical results. Stringent requirements for microbiological analysis are necessary; therefore, the use of aseptic sampling techniques and clean and sanitized equipment is of utmost importance.

The purpose of aseptically collecting a sample is to prevent contamination of the sample or the surrounding product/product contact area.



Glossary of Useful Terms

Acute – Sudden onset and repaid progression (when used in reference to a disease or condition).

Aerobic – Bacteria that require oxygen to grow or will grow in the presence of oxygen.

Anaerobic – Bacteria that do not utilize oxygen to grow, or will not grow in the presence of oxygen.

Aqueous – Relating to water.

Autoinfection – Infection caused by bacteria, viruses, or parasites persisting on or in the body.

a_W (Water activity) – The measure of free water in the environment that is available for use by bacterial cells.

Bacteriocin – A substance that is produced by specific bacteria that is toxic to closely related strains of the same specific bacteria and either kills or slows the growth of those other specific bacteria.

Coliform – Bacteria that most often inhabit the intestine of animals and do not utilize oxygen, but can grow in its presence. Bacteria that are classified as coliforms have the same shape and many of the same characteristics. These bacteria are used as indicators of sanitary quality in many food products.

Colony – A visible growth of microorganisms (bacteria) on a solid nutrient medium. Members of the colony are identical to the original or parent cell.

Colony-forming units (CFUs) – Visible units counted in a plate count, which may be formed from a group of cells rather than from one cell. CFU is used to measure the number of living cells present.

Curing – The addition of salt, sodium, or potassium nitrate (or saltpeter), nitrites, and sometimes sugar, seasonings, phosphates, and cure accelerators (e.g., sodium ascorbate), to pork for preservation, color development, and flavor enhancement.

Detection limit – The lowest threshold amount of bacteria that must be present in a sample to be found. Detection level depends upon methods used.

Direct plating – The application of a sample, or dilution thereof, to solid media, usually containing agar and other material used to grow and enumerate bacteria.

D-value – The amount of time needed to destroy one log unit of a specific bacteria at a specific temperature in a specific medium.

Enrichment – The addition of nutrient-rich broth so that certain bacteria or types of bacteria increase in number to result in a bacterial cell count that is higher than the detection limit. This is used to detect only the presence or absence of the bacteria, not the amount present.

Enterobacteriaceae – Large group of bacteria that are closely related and are commonly found in fecal material of warm-blooded animals. They include coliforms and pathogens such as *Salmonella*.

F-value – Measured in minutes, the D-value of a specific organism at 250 °F (121 °C) multiplied by the desired log reduction.

Facultative aerobes – Microorganisms that grow best when oxygen is present but do not need it to grow.

Facultative anaerobe – Microorganisms that do not need oxygen to grow, but will use it when it is present.

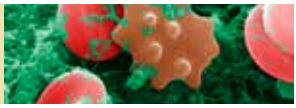
g (Generation time) – The time it takes for bacterial cell numbers to double.

Germination – The process of a spore becoming a vegetative cell.

Gravid – Pregnant or containing eggs.

Heat labile – Destroyed or altered by heat.

Hemolytic anemia – A condition in which red blood cells are destroyed and removed from the bloodstream before their normal lifespan is over. This leads to the blood having a lower than normal number of red blood cells. Hemolytic anemia can lead to many health problems, such as fatigue (tiredness), pain, irregular heartbeats, an enlarged heart, and heart failure.



Hemorrhagic colitis – Abdominal cramps and bloody diarrhea, without fever.

Inactivation – The destruction of the activity of a pathogenic microorganisms so the microorganism is no longer harmful.

Infection – The invasion by, and growth of, pathogenic microorganisms in a host.

Infectivity – The ability to produce infection.

Infestation – The presence of large numbers of organisms on or in a host causing illness or damage.

Inhibition – The slowing or stopping of bacterial growth.

k (Growth rate) – The rate at which bacterial cells reproduce.

Lag time – The time that bacteria take to become acclimated to a new environment before starting to multiply. Bacteria divide and their numbers grow exponentially: 1 becomes 2 becomes 4 becomes 8, etc.

Lethality – The effectiveness of a treatment to destroy or kill bacteria.

Log unit – An exponential (multiplicative) relationship between units in a numerical scale. The exact relationship is conveyed by the base. Assuming that "log" is "log base 10," every unit is expressed as the exponential of 10. For example, 1 log10 is equal to 10^1 (10 to the 1st power) or 10; 2 log10 is equal to 10^2 (10 x 10) or 100, and so on. Moving 1 unit in the log10 scale is equivalent to multiplying or dividing the preceding number by 10 (multiply if increasing the log number, divide if decreasing the log number).

Scientists convert bacterial counts to log scales (or plot on log-log or semi-log graphs) because it allows them to see the large changes apart from the irrelevant data inherent in the process of measuring bacteria populations. If two populations of bacteria differ by less than 10 fold (1 log10 unit), the distinction is not likely to be significant. But differences of 10, 100, or 1,000 fold (1, 2 or 3 log10 units) are more likely to be significant and scientifically important.

Mesophiles – Bacteria that have optimum growing temperatures between 77 °F (25 °C) and 104 °F (40 °C).

Microaerophiles – Microorganisms that require oxygen at a lower level than is found in normal air to survive.

Microflora – Bacteria, molds, and yeasts.

Obligate aerobe – Microorganisms that require a high concentration of oxygen to survive.

Obligate anaerobe – Microorganisms that must avoid all oxygen to survive.

Oncosphere – The larva of the tapeworm contained within the external embryonic envelope and armed with six hooks.

Pathogen – Organisms that cause illness. These organisms include bacteria, protozoa, or viruses.

pH – Level of acidity or alkalinity in a product. The pH scale ranges from 1 to 14, with 7 considered neutral, 1 the most acidic, and 14 the most alkaline. Fresh meat usually has a pH near 5.6.

Proglottid – An individual segment of a tapeworm.

Psychrotrophs – Bacteria that have optimum growing temperatures between 68 °F (20 °C) and 86 °F (30 °C), but can grow at temperatures as low as 32 °F (0 °C).

Scolex – The head of a tapeworm that usually has suckers and/or hooks.

Shocked (**heat shocked**) – Occurs when a product is heated, but the temperature is not high enough to destroy the bacteria. It results in bacteria that are injured for a while, but in most cases can repair itself and becomes more resistant to heat the next time the product is heated. Heat shocked also can refer to the process by which a spore is induced into germination. When a product is heated thoroughly, the vegetative cells are destroyed, but the spores are undamaged by the heat. The spores then germinate into vegetative cells once the temperature has decreased to an optimum level.

Significant difference – Statistical difference in results.

Spore – A highly resistant, dormant form that some bacteria can change into. Spores are usually very resistant to heat, long periods of dryness, and other adverse conditions that normal vegetative cells cannot survive. Most must be

adverse conditions that normal vegetative cells cannot survive. Most must be heat-shocked to germinate into normal, vegetative cells. Most of the time, spores have a toxin associated with them, either within the spore covering, or released at the time of germination, or when becoming a spore (sporulation).

Strain – A specific subset of bacteria. For example, ***Escherichia*** is the genus, ***coli*** is the species, and **O157:H7** is the serotype (strain).

Thermotolerant – Bacteria that can withstand higher-than-normal temperatures.

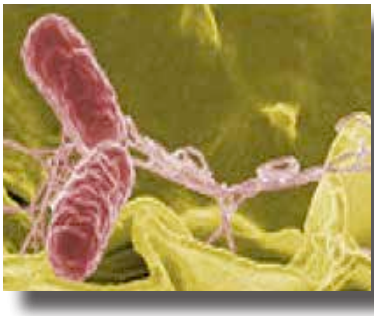
Thermobocytopenia – An abnormal drop in the number of blood cells, called platelets, that are involved in forming blood clots.

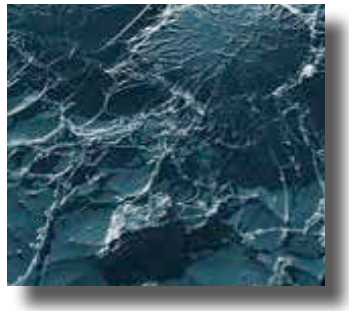
Toxin (**enterotoxin, mycotoxin, neurotoxin**) – A compound produced by a bacterium or fungi (molds and yeasts) that can cause illness in other living organisms. Specific examples include enterotoxins which affect the intestine, mycotoxins (toxins produced by fungi), and neurotoxins that attack the nervous system.

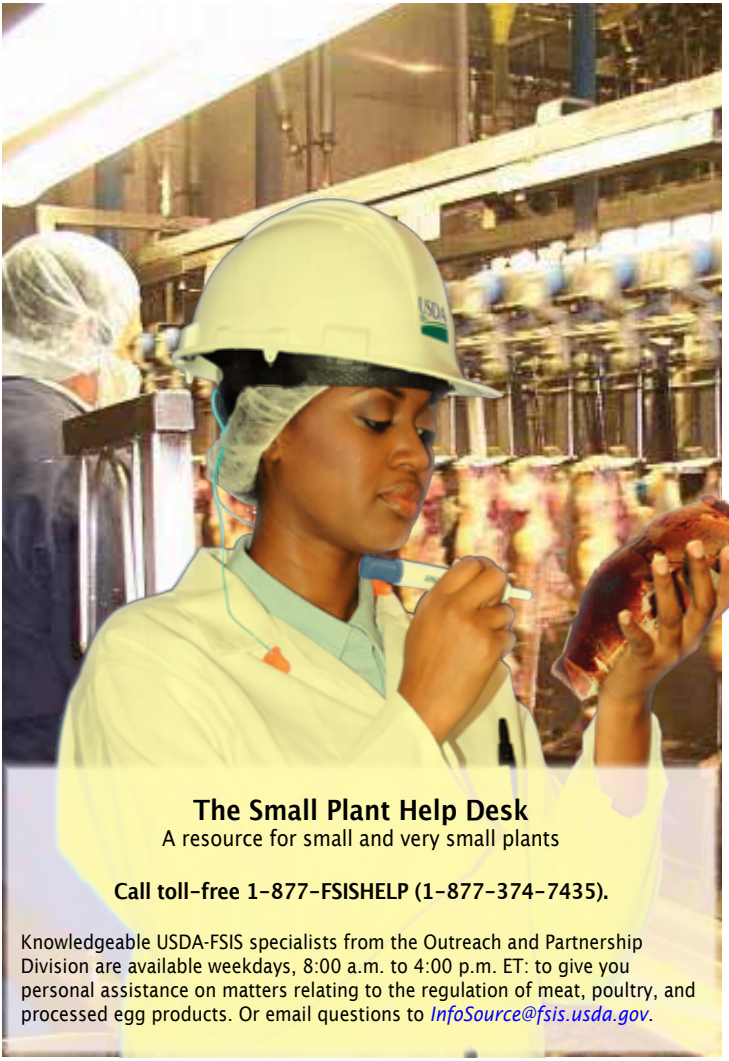
Transdermal synergists – Compounds that work with other compounds against bacteria when applied to the surface of a carcass.

Treatment – The method of processing that is being tested. A good research study will compare various treatments, such as levels of salt in a product, to a control. In this example, the control may be no salt added. All other conditions should remain the same for all samples tested, except the specific treatment.

Vegetative cell – The normal bacteria cell. This is in contrast to a spore. Vegetative cells are susceptible to destruction or damage from heat, additives, and other factors that can damage and destroy them relatively easily.







The Small Plant Help Desk

A resource for small and very small plants

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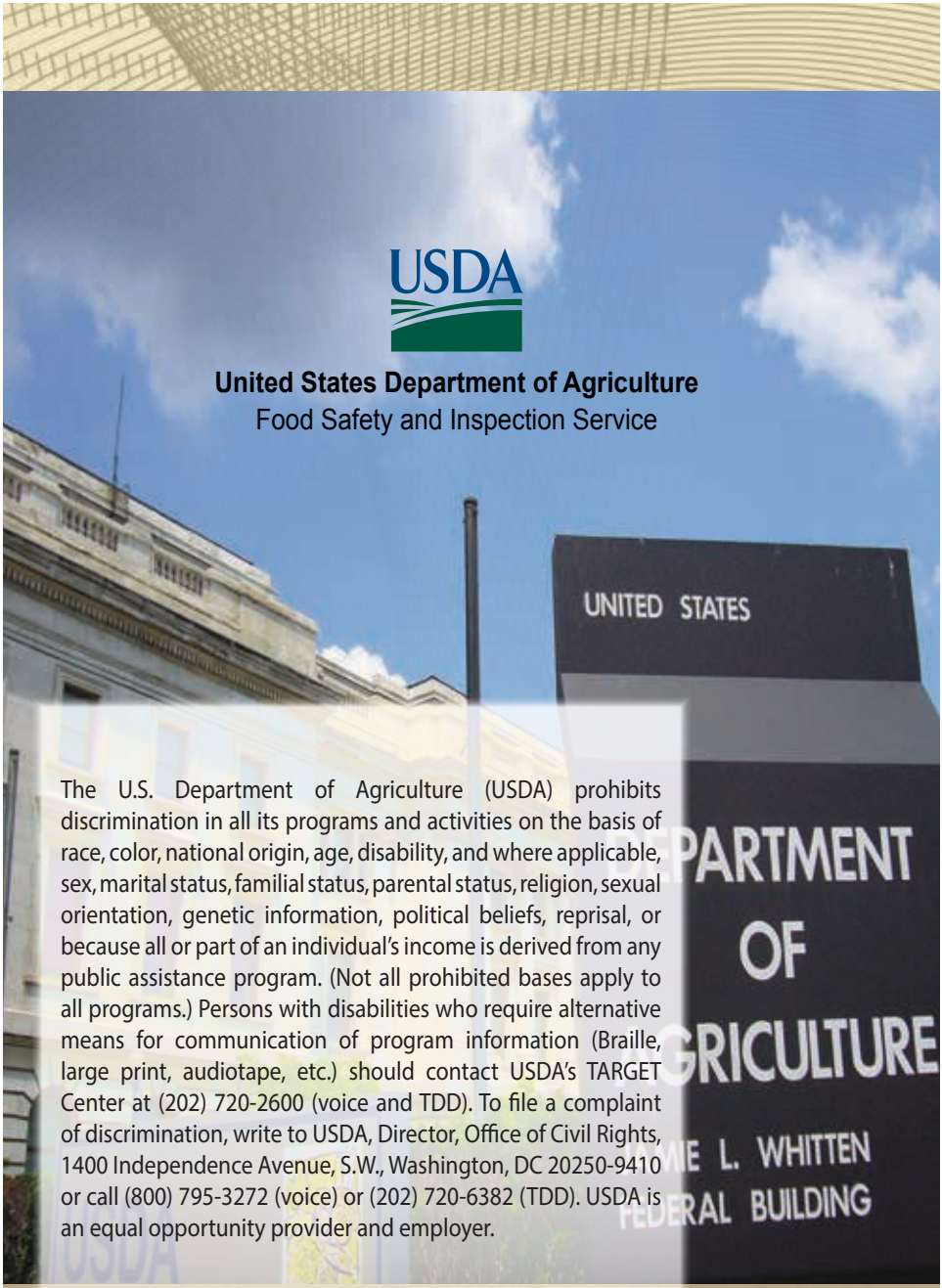
Knowledgeable USDA-FSIS specialists from the Outreach and Partnership Division are available weekdays, 8:00 a.m. to 4:00 p.m. ET: to give you personal assistance on matters relating to the regulation of meat, poultry, and processed egg products. Or email questions to *InfoSource@fsis.usda.gov*.



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