





SECTION 2

FOOD MICROORGANISMS		
Pathogen	Hazard	Control
 Parasites	<i>Trichina</i> spp., <i>Anisakis</i> , beef tapeworm, <i>Toxoplasma gondii</i> Live within animals and fish (in muscle, intestinal tract): 1 to 10 will cause illness.	Reduced to safe level by cooking to 145°F, 15 seconds. Killed by freezing, -4°F, 7 days.
 Molds	Most molds in kitchen are spoilage. <i>Aspergillus</i> spp., <i>Fusarium</i> , penicillin grow on stored grain and peanuts. They can form aflatoxins and can cause cancer.	Cut off / throw away moldy food. Keep grains and nuts dry. Government testing controls
 Viruses	Noroviruses, hepatitis A, rotavirus. Source is human feces and vomit.	Double wash fingertips after using the toilet.
 Bacteria	Vegetative bacteria: <i>Salmonella</i> , <i>E. coli</i> , <i>Vibrio</i> , <i>Shigella</i> , <i>Streptococcus</i> , etc. Spores: <i>Clostridium perfringens</i> , <i>Bacillus cereus</i> , <i>Clostridium botulinum</i> in hot food survive pasteurization as spores (dried hibernating vegetative cells).	Reduce to a safe level with pasteurization, 100,000-to-1; 145°F, 3 minutes; 150°F, 1 minute; 155°F, 15 seconds OR Double wash fruits and vegetables (100-to-1 reduction). Hold ≥135°F / >130°F roasts to prevent outgrowth. Cool 135 to 41°F, 6 hours <1 gallon / <2 inches deep). Cold hold 41°F.

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Food Microbiology

Understanding how and why pathogenic microorganisms in food cause foodborne illness makes it possible to use HACCP to anticipate and control contamination and growth of pathogens.

Microorganisms are so small that the individual cells cannot be seen without a microscope. There are four classes of microorganisms: bacteria, viruses, yeasts, and molds. Yeasts are really not a food safety problem and are not discussed in this text. Parasites are micro- or macroorganisms that exist at the expense of the host.

Bacteria

Bacteria are composed of one cell. They are classified according to their shape: bacilli (rod), cocci (round), and spirilla (spiral). Some bacilli form spores that are very heat resistant and survive pasteurization cooking. Examples include *Clostridium* spp. and *Bacillus* spp. They are in food when it is taken home as leftovers or as take-out food. To inactivate spores, one must heat the food in a can or jar in a pressure retort to 250°F for 3 minutes. This makes the food commercially sterile.

Viruses

Viruses are not true living cells and are much smaller than bacteria. A virus is composed of a protein coating around DNA and RNA material. To multiply, viruses must use compounds from other living cells. For example, the hepatitis A virus uses material from human liver cells to reproduce. Typical illnesses caused by viruses are colds, flu, hepatitis, and Norwalk gastroenteritis. Viruses are difficult to inactivate with sanitizers and heat. Since the only sources of this hazard are the feces and vomit of others, double hand washing after using the toilet is the BEST control.

Molds

Some molds produce mycotoxins such as aflatoxin (a nerve poison). They can also cause allergic reactions in people. Molds appear as cottony, powdery, or fuzzy tufts and patches. Most molds have a pungent odor. Molds can grow above 14°F during frozen storage. They grow in acid foods such as salad dressings, tomato ketchup, salsa, and fruit drinks.

The presence of mold on products is an indication of poor farm practices, poor sanitation, poor refrigeration, and/or over-extended shelf life. If, for example, a cheese is moldy with a white, fuzzy mold, the moldy portion can be cut off (½ inch below the mold) and thrown away. It is important not to touch the mold in order to prevent it from spreading to other food. While molds can be a quality and a minor food safety issue, the FDA does not mention molds in the model food code or food safety rules.

Parasites

Parasites are organisms that live at the expense of some other living cell, plant, or animal (including humans). Parasites of major concern in foods are tapeworms found in fish, trichinae found in pork, and *Giardia* found in water and feces. Although bacteria, viruses, yeasts, and molds are not effectively destroyed by freezing, parasites can be destroyed if food containing them is frozen. The FDA and USDA have specified temperatures at specific times, depending on the type of parasite and the size of the product. The colder the temperature, the more rapid the destruction. For example, -4°F for 7 days is an effective control. Parasites are also destroyed when food is heated to 145°F for 15 seconds or above for a time sufficient to kill *Salmonella* spp.

THE BIOLOGICAL HAZARD

- All food has pathogenic and spoilage organisms. They come on food, people, water, air, and insects, and multiply during processing.
- They multiply well in most of the foods that we normally eat.
- Cooking only reduces the number of vegetative microorganisms, activates spores, and does not touch toxins and poisons.
- During warm holding and slow cooling, spores outgrow, and cell population increases rapidly.



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Purchasing Food

No USDA, FDA, or commercially inspected raw food can be assumed to be safe. To assure food safety, food must be purchased from suppliers who can identify the level of pathogenic substances in their food and certify the safety of their product(s). The government inspection process of raw food does not control the microbiological, chemical and physical hazards in food that are known to produce foodborne illness. The number of pathogens present in the food must be below a level that will make consumers ill. The food must then be received, stored, prepared, and served at temperatures within times that keep microbial growth, particularly that of toxin-producing microorganisms, to less than 10 generations (to multiply 10 times, or increase by a factor of 1 to 1,024). The FDA Food Code allows enough time at 41°F (7 days) and 100°F (4 hours) for 10 multiplications of pathogens. If the food is cooked, it should be heated sufficiently to reduce salmonellae from 10,000,000 to 1 per gram.

Microbial Contamination

Raw, refrigerated, and frozen foods are contaminated with many spoilage microorganisms and some pathogenic microorganisms when delivered. The normal load of spoilage microorganisms on food items varies from several thousand to millions of bacteria per gram. (There are 28.4 grams per ounce.) Hamburger, chicken, fish, and vegetables often have at least a million spoilage bacteria per gram by the time these food products are cooked for service.

Temperature Influences: Multiplication

The biological hazard in foodservice is the ability of pathogenic microorganisms to multiply (grow): in raw food during refrigerated holding; in food during slow cooking (more than 6 hours to 130°F), in cooked food during slow cooling, or holding of food at 85 to 120°F for more than 2 hours. [Note, the FDA recommends that food should be cooled from 135 to 70°F within 2 hours, followed by further cooling to 41°F (6 hours, total time)]. USDA Guidelines for cooling recommend continuously cooling food, within 90 minutes after cooking, from 120°F to 55°F within 6 hours, followed by further cooling to 40°F (no time limit) before boxing.)

Growth or multiplication of pathogenic bacteria in food during these hazardous procedures may be sufficient to cause illness when the food is consumed. The optimum temperature range for rapid pathogen multiplication (more than once an hour) is 85 to 120°F. At 95 to 115°F, doubling in population can occur every 8 to 25 minutes. Pathogen growth slows as the temperature approaches the high temperature growth limit (125°F for *Clostridium perfringens*) or low temperature limit of 30°F for *Yersinia enterocolitica* and *Listeria monocytogenes*. Therefore critical temperatures for food storage or holding to prevent pathogenic microorganism growth are less than 30°F and higher than 125°F.

Reduction of Microorganisms through Cooking

Cooking in foodservice only pasteurizes the food, that is, reduces pathogenic vegetative microorganisms in the food to a safe level. Cooking has essentially no effect on spores, toxins, and poisons.

HOW DO YOU KNOW IF FOOD IS HAZARDOUS OR SAFE?

You control the process.

Food Spoilage Microorganisms

Do not cause illness.
Change the flavor, odor, and appearance of food.
Inhibit growth of pathogens.

Food Process Microorganisms

They "spoil" / ferment the food. Do not cause illness.
Used in the production of food products
(e.g., vinegar, bread, sauerkraut, cheese).

Food Pathogens

Cause illness.
Often do not change the flavor, odor, and appearance
of food to indicate that the food is hazardous.
If in doubt about how food was handled after cooking,
throw it out.



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food) that cause illness have no effect on the taste, odor, or appearance of the food. Many people even say that the food such as roast beef, cake icing, tuna salad, or other food that made them sick was the best they had ever tasted. The old rule, "**If in doubt, throw it out,**" must be followed. Doubt, though, cannot be based on odor, appearance, or taste. Doubt is measured by knowledge about whether or not the food was handled and stored correctly. For example, food found in the wrong place, at the wrong temperature must be thrown out. It should not be tasted. It could kill you.

Potentially hazardous foods that are involved in foodborne illness outbreaks include: meat, fish, poultry, dairy products, baked products, eggs, infant foods, candy, vegetables and fruit, Chinese food, salads, sandwiches, and beverages. As this list indicates, any food can become hazardous if not purchased from a safe source and if not prepared, stored, and handled properly.

Food Spoilage Microorganisms

Food spoilage microorganisms are good in HACCP and do not cause illness. Excessive, prolonged growth of spoilage microorganisms changes the odor, flavor, and texture of the food so much that it becomes unpalatable. If the growth of spoilage microorganisms is minimal, spices can be used (as they have been used for thousands of years) to cover up the effects of spoilers so that the food can be eaten.

In raw food, spoilage microorganisms are usually present in much higher numbers than pathogenic bacteria, and are able to grow more rapidly at temperatures below 80°F. Hence, spoilage microorganisms that often produce acid in many cases are able to stop or inhibit the growth of pathogens by a process called competitive inhibition. Competitive inhibition occurs when spoilage bacteria and pathogenic bacteria compete with each other for nutrients in food in order to multiply.

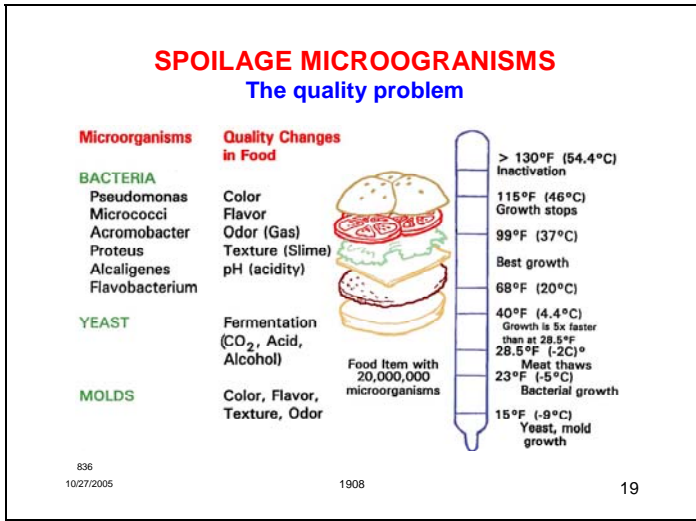
When raw food is cooked above 160°F for 15 seconds, vegetative pathogenic microorganisms and most of the spoilage microorganisms are destroyed. The food is safe but the spoilage competitive microorganisms are gone. This makes the cooked food far more vulnerable to a few pathogens on hands or from cutting board cross-contamination of cooked food. With correct temperatures and no competition from spoilage microorganisms, the contaminating pathogens grow well. Temperature abuse also allows spores to outgrow into vegetative cells.

Food Process Microorganisms

The food process microorganisms that are used to produce many food products such as beer, sour cream, sauerkraut, wine, bread, cheese, soy sauce, and salami actually make the food safe. Bacterial counts become very high – 100,000,000 or higher. The growth of food process microorganisms produces by-products such as acids (lactic and acetic), carbon dioxide, and alcohol, which help preserve the food and destroy the pathogens. When these same microorganisms are allowed to grow in foods where their presence is not desired, they are called spoilage microorganisms.

Food Pathogens

How can the safety of food be verified? Food safety cannot be judged by the taste or smell of the food. Most food pathogens (pathogenic bacteria, viruses, yeasts, molds, and parasites in



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Balancing Spoilage Organisms and Pathogens

Most food, especially raw food, which is eaten every day, contains thousands to millions of spoilage microorganisms per gram. Some of these microorganisms are critical to keeping a healthy intestinal microflora and maintaining a high infection resistance in the body. Actually, it appears that people should eat a mixture of spoilage microorganisms to stay healthy. However, it is also essential to keep pathogens in food at low levels to assure food safety.

Spoilage Microorganism Growth Conditions

Spoilage microorganisms are the real problem in terms of customer satisfaction. They begin to grow when food is still frozen. Yeasts and molds start to grow at 15°F. Spoilage bacteria begin to grow at 23°F. They actively grow at 28°F when meat, poultry, and fish are thawing. Spoilage microorganisms grow best at 70 to 80°F and are inactivated at temperatures of 115°F and above.

Normal Spoilage Organism Contamination

When fresh foods are received, they have a mixture of spoilage microorganisms. These include bacteria such as *Pseudomonas*, *Achromobacter*, *Micrococci*, *Proteus*, *Alcaligenes*, *Flavobacterium*, yeasts, and molds. The growth of these microorganisms produces the typical changes in food associated with signs of spoilage. These changes include color change, slime, odor, flavor change, and texture change. Fermentation of sugar occurs and CO₂, acids, and alcohol are produced.

The Importance of Refrigeration at 28 to 32°F

Foodservice refrigerators and coolers used for meat, poultry, and fish product storage should be kept at 28 to 32°F in order to slow the growth of spoilage microorganisms and prevent pathogenic bacteria growth. Ground beef spoils 3 to 5 times faster at 45°F than it does at 32°F.

For example, average quality ground beef with 500,000 spoilage bacteria per gram upon delivery on Friday, if stored at 45°F, will have 360,000,000 spoilage bacteria per gram and obvious off-flavor and slime by the following Monday at noon. Spoilage microorganisms multiply once every 7.8 hours at 45°F, so between Friday and Monday there would have been 9.5 generations of growth. On the other hand, if the ground beef is kept at 32°F, there will only be 2.5 generations of growth. This means that there will only be 3 million bacteria per gram by Monday, and the hamburger will be quite acceptable. Maintaining storage temperatures of 28 to 32°F is also important for raw poultry and fish since they are just as contaminated with spoilage microorganisms as ground beef.

TYPES OF ILLNESS

FOOD POISONING: Vomiting and illness in 1 minute to 4 hours resulting from eating a substance or compound that the body cannot detoxify [e.g., some mushrooms, cleaning and sanitizing chemicals, MSG, sulfites, metal (lead) poisoning; also, scombroid fish poisons (histamine) and ciguatera toxin from dinoflagellates in tropical waters].

Normal cooking has no effect on poisons and will not make poisonous food safe. If in doubt, throw it out.

FOOD INFECTION: Diarrhea and vomiting in 6 hours to over 2 weeks as a result of consuming food containing living pathogenic microorganisms that then multiply in the body [e.g., *Salmonella* spp., hepatitis A virus, Trichinae].

Normal cooking and control of cross-contamination makes food safe to eat.

FOOD INTOXICATION: Vomiting and illness in 15 minutes to 4 hours caused by consuming food containing toxins produced by bacteria when they multiply in food [e.g., *Staphylococcus aureus*, *Clostridium botulinum*, *Bacillus cereus*].

Normal cooking will not always inactivate the toxins and make food safe. If in doubt, throw it out.

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Types of Foodborne Illness

In order to control hazards, it is important to know what illnesses the hazards produce. The major types of foodborne illness are:

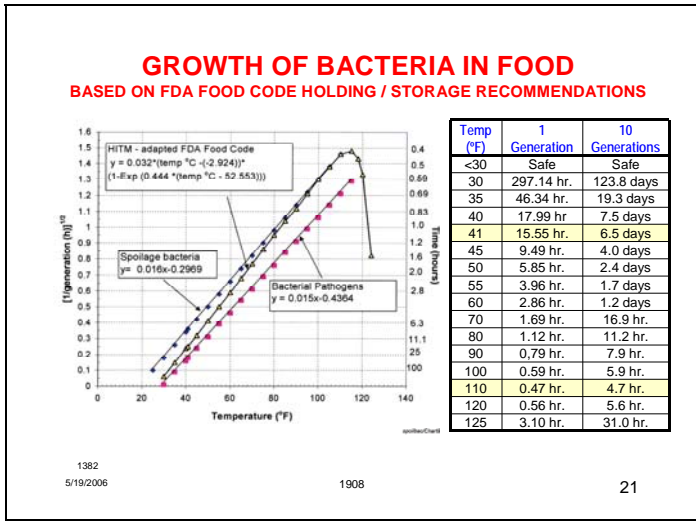
- **Food poisoning.** This is illness that results from eating a substance that the body cannot detoxify (e.g., cleaning chemicals, MSG, sulfite, metal poisons, and some mushrooms). If these toxic compounds or chemicals are on the surface of the food, they might be washed off. If they are inside the food, nothing can be done to get rid of them. Heating has no effect on poisons. In order to avoid this problem, food should be purchased from a source that certifies safe levels of poisonous substances. The food supplier should know and control the types of chemicals used during growth and production of the food. In the foodservice facility, cleaning and pesticide chemicals should be locked up and kept separate from food supplies. Only the amount and kind of preservatives and other culinary chemicals allowed by law should be added to food.
Ciguatera fish poisoning is the most common type of seafood poisoning caused by eating reef fish such as barracuda, snapper, eel, grouper, and amberjack are most likely to cause this type of poisoning. Scombroid poisoning is the second most common type of seafood poisoning, resulting from eating spoiled / decayed fish, particularly due to improper handling during storage or processing. It is often associated with fish such as mackerel, tuna, mahi-mahi, and anchovies.
- **Food infection.** This is illness that results from eating food containing live pathogenic bacteria (e.g., *Salmonella* spp.) or viruses (e.g., hepatitis) that multiply in the body. A few of some pathogens, 10 to 100 in a meal, can cause illness. They generally come from the feces of humans or animals. When fields are fertilized with animal manure, when animals are slaughtered, when humans use the toilet and get feces on their fingertips, infective microorganisms are transferred to the food. Ten to 100 cells or virus particles in the serving of food can make people ill. If a healthy person with a good immune system wants to eat uncooked meat, poultry, fish, etc., then, to be safe, he/she must know that animal and human fecal contamination is so low, due to careful processing of the raw food, that they will not be made ill. It is up to the grower-

harvester and supply system to certify that these foods have a low pathogen count and that they have been handled in a way that keeps their pathogen count below a threshold that causes illness.

Immune-deficient individuals such as children less than 5 years old, older people, people on antibiotics, and cancer and Aids patients should not eat raw meat, poultry, fish, or shellfish, because low levels of infective microorganisms could be dangerous.

When food contaminated with pathogenic microorganisms that produce foodborne infections is given an adequate time-temperature pasteurization, for example, at 150°F (1 minute) / 155°F (15 seconds), these microorganisms are destroyed and the food is safe to consume. Food containing hepatitis A virus must be heated to temperatures above 180°F. Viruses are best controlled by washing hands.

- **Food intoxication.** This is illness caused by eating food containing toxins produced by bacteria (e.g., *Staphylococcus aureus*, *Clostridium botulinum*, and *Bacillus cereus*) that have multiplied in a food or food product normally after cooking. The only control is to prevent the multiplication of these microorganisms in the food. The toxins (waste products from the multiplication of these microorganisms) will not be inactivated by ordinary reheating to 165°F, and once these toxins are present in food, the food becomes hazardous. To prevent this type of pathogen multiplication and toxin production and to assure that food is safe, when food is cooked, it must be heated to higher than 130°F in less than 6 hours to prevent multiplication during heating. After pasteurization, food must be held at higher than 130°F (the FDA Food Code hot holding recommendation of 135°F is 5°F higher than necessary), or cooled from 135 to 70°F within 2 hours, followed by further to 41°F (6 hours or less, total time), according to the FDA Food Code. (USDA Guidelines recommend continuously cooling food, within 90 minutes after cooking, from 120 to 55°F within 6 hours, followed by further cooling to 40°F (no time limit) before boxing.) The FDA food code recommends that food be stored at 41°F or less after cooling or preparation and consumed within 7 days. The FDA code also allows time as a control, and prepared food can be at any temperature from 41 to 135°F for 4 hours, if leftovers are destroyed.



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Growth of Foodborne Disease Bacteria

Bacterial growth conditions. For optimal growth, bacteria require the proper environmental conditions of temperature, adequate nutrients, free water, time, and the correct pH (greater than 4.6). Any imbalance of these factors can limit bacterial multiplication. Bacteria also require a proper atmosphere (proper amount of oxygen). Some bacteria are aerobic (i.e., require oxygen), some are anaerobic (i.e., require no oxygen) and some bacteria are facultative and can survive in atmospheres with and without oxygen. Any imbalance of these factors can slow down bacterial multiplication.

Growth Process

If the right conditions exist, bacterial growth occurs. There is a short period of time, however, after the correct growth conditions are met before bacteria begin to multiply. This is known as the "lag phase". During the lag phase, the bacteria adjust to the environment, and the metabolic cycle within the cells gradually begins to function. The lag phase can last for a few minutes to days. Its length is determined by the temperature, atmosphere, water, and pH of the surrounding environment. Normally it takes at least 2 hours for a spore to outgrow in cooked, improperly cooled food and begin multiplying at 110 to 120°F. After the lag phase, the bacteria begin to multiply logarithmically: 2 become 4, then 8, then 16, and so on.

Pathogens causing foodborne illness that grow at the lowest temperature include vegetative cells of *Listeria monocytogenes* and *Yersinia enterocolitica*. These pathogens are commonly found in soil and water. They begin to multiply at 29.3°F. *Listeria monocytogenes* is responsible for stillborn infants when pregnant women become infected with this pathogen. The table in the above illustration is a list of the safe times for storing food at specified temperatures before it should be eaten or cooked in order to limit the multiplication of *L. monocytogenes* and other foodborne bacterial pathogens.

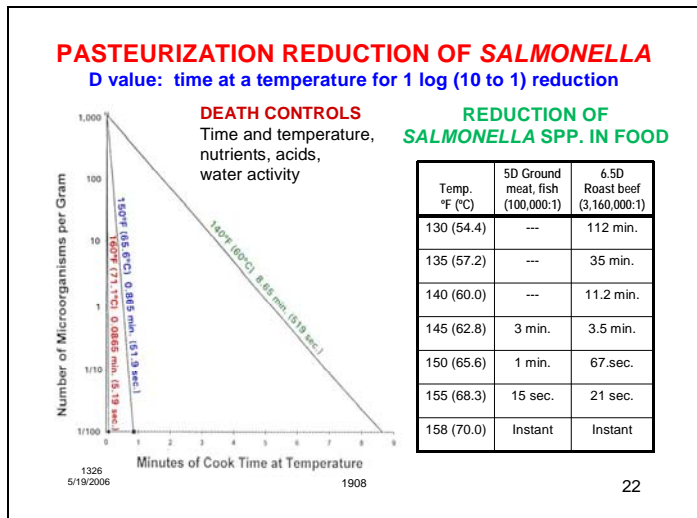
Bacteria enter the stationary phase when there are so many (about 10,000,000 to 50,000,000 per gram) that growth is stopped. At this point, the supply of nutrients in the system is used up, and the population has produced a substantial amount of waste. This is the point at which food becomes slimy. Slime is simply layers of billions of bacteria, one on top of one

another. The death phase occurs after this stationary phase when bacterial enzymes from the waste products dissolve the non-growing bacterial cells.

One of the most serious mistakes food handlers make is to add old product to fresh product. This gives a large population of microorganisms in the old product a fresh supply of nutrients in which they can begin to multiply, causing the fresh product to spoil rapidly and pathogens to grow.

The table and graph above are based on the FDA Food Code recommendations for storing food and the growth limits of bacterial pathogens. Bacterial pathogens that produce foodborne illness begin to multiply at 30°F, but do not multiply above about 125°F.

The FDA Food Code recommends that ready-to-eat food be held at 41°F or below for no longer than 7 days, or no longer than 4 hours if held between 41 and 135°F. The author has applied bacterial growth mathematics to develop the maximum holding times at various temperatures as shown in the table. Compared to actual pathogenic bacteria multiplication, the government-based recommendations are quite safe.



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Destruction of Bacteria

Time and temperature can be manipulated in order to destroy bacteria. Just as growth is logarithmic, destruction is also logarithmic. The higher the temperature, the shorter the time required to accomplish destruction of bacterial cells and spores. The figure above shows data that are typical of *Salmonella* spp. *Salmonella* spp. is a common foodborne illness-producing organism and hence, is suitable to use to develop safety standards. One thousand *Salmonella* spp. per gram can be reduced to 1 per 100 grams of food (a 5D or 5 log reduction) in 8.7 minutes at 140°F. (A 1D reduction is 1 log reduction.) At a temperature of 150°F, the process would take 1 minute, and at 155°F, 15 seconds. At 158°F, death is considered to be instant. Only 5.2 seconds would be needed to accomplish the same reduction at 160°F. Note that for each 10°F increase in temperature, the salmonellae die 10 times faster. Time and temperature control is far more important in pasteurization of food than in cold holding and refrigeration; 3°F error in measuring the coldest spot in a food item can mean the survival of twice as many organisms. Precise cooking is essential if there is to be safety without overcooking.

Roasts can be made safe at 130°F with a 112-minute hold. This is needed, because consumers demand rare prime rib. However, for chops, ground meat, and fish, the lowest temperature and time allowed is 145°F, with 3 minutes hold. This is based simply on the difficulty of holding a food in the fryer or grill for a fixed time. Therefore, rare meat, unless the supplier certifies it as vegetative-pathogen free, cannot be done safely except in slow cooking, such as oven roasting of beef. Note, a hamburger, meatball, or other highly contaminated food can be made safe by holding the center temperature of the food at 145°F for 3 minutes or 150°F for only 1 minute. In ordinary cooking, since the outside of the food is well above 150°F, by the time the food is plated, if the item is taken off the grill at 150°F center temperature, it will have spent more than 1 minute above 150°F and will have a 100,000-to-1 kill. If the hamburger is ground from fresh product, not aged beef, and if there are no additives such as soy, the color will be a pleasing, medium pink at 150°F center temperature. When meat is heated to 160°F center temperature, there will be little red color left in fresh beef, pork, lamb, chicken, etc., and these items, which are medium well, are safe. Note, both the USDA and FDA prohibit cooking to color

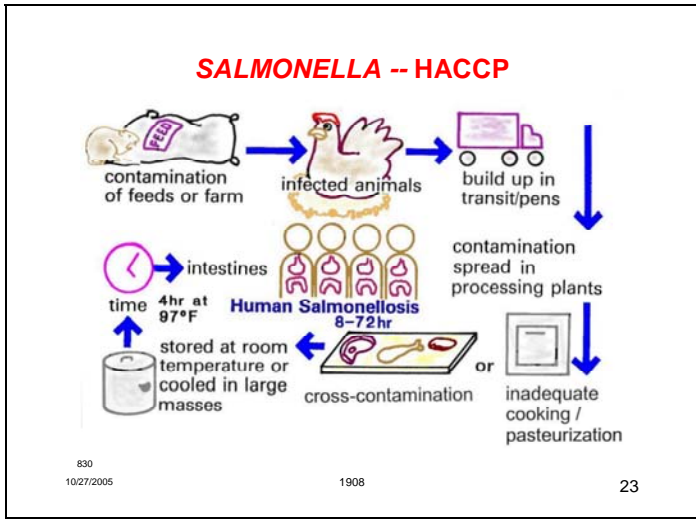
or until juices run clear for verification of a safe cook. A tip-sensitive thermometer must be used. Cooks have been taught to cook meat until the bright red color is gone, not because of safety, but rather, it was assumed that no one had a proper thermometer to measure temperature of the meat. Thus, color change of the meat to grayish brown color was considered to be an accurate indication of adequate cooking time and temperature

The addition of lemon juice, vinegar, and wine to food products not only adds flavor to the product but also lowers the pH of the environment by providing acids, which slow down or inhibit bacterial growth and aid in destruction of bacteria when combined with heat. It will also turn meat brown at lower cooking temperatures. If it is aged and acidic, it is brown at 150°F. Meat with 15% textured vegetable protein is brown at 140°F. On the other hand, if meat is cooked with onions and celery, high in nitrate, the meat becomes cured and does not turn completely brown, even at 180°F.

Water Activity

Microorganisms require moisture to be destroyed easily. If the amount of water is restricted, destruction will take 10 to 100 times longer. Moist heat (steam or heating in water) destroys vegetative bacterial cells and spores on the surface of food more rapidly than dry oven heat.

When salt, sugar, and fat are added to food products, these ingredients bind water and make the destruction of vegetative bacteria more difficult. Foods containing higher amounts of salt, sugar, or fat, such as egg custards, need to be cooked to temperatures that are 10°F higher than those for *Salmonella* shown above, or should be pasteurized 10 times longer than normal to get the same inactivation of the bacteria present in the food.



that *Salmonella* spp. will not be transferred from one food (e.g., raw poultry) to another food (e.g., cooked chicken or celery that is to be used in a salad).

Employees must wash hands frequently when working in food production facilities and use hand washing methods that prevent the cross-contamination and transmission of *Salmonella* spp. and other pathogens in their feces to food.

Infection Characteristics

The vegetative cells cause illness by multiplying in the intestinal tract. The illness is characterized by diarrhea, vomiting, fever, chills, and cramps. Because it is an infection, the presence of only a few cells can cause illness. The illness develops within 12 to 36 hours of eating food containing sufficient numbers of microorganisms. The illness can go on for a number of days or weeks, causing dehydration of victims and possibly causing death in the elderly and infants.

The number of microorganisms needed to cause illness varies with the type of *Salmonella* spp. species and the susceptibility or resistance of the host. As few as 1 or 2 viable cells in a meal for infants and immune-compromised individuals can make them ill, while healthy individuals might consume 100,000 to 10,000,000 viable cells in a meal without becoming ill.

Salmonella spp. Food Infection

There are over 2,000 strains of *Salmonella*. All species and strains are pathogenic to humans. *Salmonella* spp. are found in the intestinal tract of infected animals and people. A variety of raw and processed foods have been found to carry *Salmonella* spp. Raw meat and poultry, shellfish, eggs and egg products, processed meat, and dried milk and cheese made from unpasteurized milk have been common sources of this microorganism. However, watermelon, cantaloupe, and fresh tomatoes have also been sources of *Salmonella* foodborne illness outbreaks in recent years.

Salmonella spp. grows in the presence or absence of air. *Salmonella* spp. grows well on or in food with a neutral pH of 6 to 7 and will multiply in food down to pH 4.1. These pathogenic bacteria can multiply on green tomatoes. However, organic acids (citric and/or acetic) found in commercial salad dressings and mayonnaise, at pH below 4.0, prevent multiplication and cause destruction *Salmonella* spp in these products.

Salmonella spp. Transmission

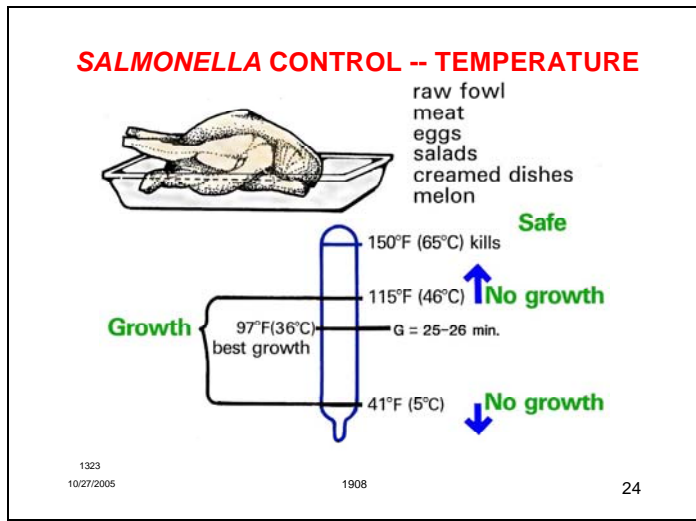
Many foods can become contaminated with *Salmonella*. Vegetables and melons become contaminated when: animal manure or human fecal material is used to fertilize the fields; contaminated water is used to irrigate crops; and people who harvest these crops are ill and transfer the bacteria to the vegetables and melons when they touch them.

Animals are infected with *Salmonella* spp. by the feed they eat or from the farm environment in which they raised. The infection spreads to other animals during transport. *Salmonella* spp. are transmitted from animal feces to carcasses and meat by equipment used in processing and by workers who touch the raw carcasses. Cross-contamination between clean carcasses and contaminated carcasses occurs in this way.

Salmonella spp. enter foodservice establishments on raw animal products or in the feces and on the fingers of infected employees. *Salmonella* spp. grow in food products when they are mishandled, undercooked, or recontaminated after cooking and then allowed to remain at dangerous temperatures.

Transmission Prevention

To prevent cross-contamination, always clean and sanitize cutting boards and knives after each food product is prepared so



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Salmonella spp. Control

An effective way to control *Salmonella* spp. is to store and prepare food at temperatures that minimize its growth, and/or to cook the food or food products to a temperature that will destroy the microorganism.

Up to 30% to 40% of all raw meat and poultry is randomly contaminated. Usually, the contamination is too low to make us ill (less than 10 microorganism per gram). But occasionally a highly contaminated item comes from the wholesale system.

There is no way to tell which item is the dangerous one. Washing meat and poultry only reduces bacteria by an insignificant number and will not really help as a safety control procedure. This practice contaminates the sink and kitchen with *Salmonella* spp. On the other hand, it is critical to double wash all fruits and vegetable to reduce contamination to a low level.

Multiplication (growth) of *Salmonella* spp. occurs optimally at 97°F, where it multiplies every 25 to 26 minutes. It begins to multiply at 41°F with a generation time of less than 3 days. At 50°F one generation time is 20 hours, and at 60°F the generation time is 6 hours. It stops multiplying at 115°F. If food is stored at less than 32°F, the temperature used to control *L. monocytogenes*, *Salmonella* spp. growth will be controlled.

Salmonella spp. is inactivated in the wet environment of at rates given in the following table. These temperatures and times can be used as a guide for inactivation of *E. coli* in hamburger.

Temperature	Ground Meat and Fish 5 D (100,000:1) kill	Roast Beef 6.5 D (3,160,000: 1) kill	All Poultry
130°F	86.2 min.	112 min	
140°F	8.6 min	11.2 min	
150°F	51.6 sec.	67 sec.	
160°F	5.2 sec	6.7 sec.	
165°F			15 sec.

Extreme care should be taken not to hold foods at the fast-multiplying temperatures of 80 to 110°F. After foods are cooked, they should be cooled from 135 to 70°F within 2 hours, followed by further to 41°F within 4 hours (6 hours total), according to the FDA Food Code. [USDA Guidelines recommend continuously cooling food, within 90 minutes after

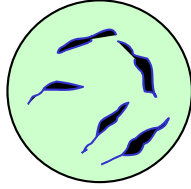
cooking, from 120 to 55°F within 6 hours, followed by further cooling to 40°F (no time limit) before boxing.]

Salmonella spp. can multiply down to a pH of 4.1. If a food product is made with raw unpasteurized eggs, it must be assumed to be contaminated with *Salmonella* spp. The pH of these food products must be adjusted with lemon juice, vinegar, or other suitable organic acid to prevent the multiplication of *Salmonella* spp. In fact, mayonnaise and other salad dressings normally have a pH of 3.5 to 3.8. After these products are prepared commercially, the mayonnaise and dressings are stored at room temperature for 3 days. During this time, the acid inactivates (destroys) the *Salmonella* spp. and other vegetative pathogens and makes these products safe.

Correct cleaning and sanitation of equipment and hands after handling raw animal products must be practiced to prevent the cross-contamination to cooked food that has few spoilage microorganisms and is very vulnerable to pathogen growth.

CHARACTERISTICS OF *CAMPYLOBACTER JEJUNI*

- Grows best in small amount of air (oxygen).
- Grows between 86°F and 113°F.
- Survives chilling and freezing temperatures.
- Source is infected animals, birds, reptiles, and people.
- Common contaminant of raw foods of animal origin (poultry, pork, raw milk).
- Vegetative cells multiply in intestinal tract to cause illness.
- Infective dose = 400 to 500 cells in a portion of food.
- Vegetative cells killed by cooking.



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Campylobacter jejuni - Characteristics Bacterial Characteristics

Campylobacter jejuni is a gram-negative, slender, curved to spiral rod that is motile by means of a single polar flagellum. It is an obligate microaerophile and grows optimally in an atmosphere containing 5-10% oxygen. It is relatively fragile and sensitive to environmental stresses of more than 21% oxygen, drying, heating, sanitizers, and acidic conditions. These microorganisms can survive refrigeration and freezing temperatures for a limited period of time.

Source

The nature and significance of *Campylobacter* spp. as a cause of illness in humans have been considered only recently. Since 1972, microbiological methods of isolating *Campylobacter* spp. have been developed. *C. jejuni* is now recognized as a common cause of gastroenteritis in humans.

It is commonly found as a pathogen in cattle, sheep, fowl, swine, and rodents. Incidents in which *C. jejuni* has been isolated as causing illness have resulted from the consumption of raw milk, undercooked poultry and pork. *Campylobacter* spp. can be spread by a contaminated water supply, and is carried by common household pets (particularly cats and dogs in poor health).

The presence of *C. jejuni* is high in fresh meat and may be as high as 100% in fresh poultry. The numbers of CFU (colony forming units) may vary from 10,000 CFU on a chicken wing to less than 1 CFU/cm² in raw pork and 1 to 10 CFU/cm² in raw beef (Genigeorgis, 1986)

Growth Conditions

Temperature. *C. jejuni* has been inappropriately termed thermophilic. It is neither thermophilic nor heat resistant. Doyle (1988) states that *C. jejuni* will not grow below 86°F. In 1981, Doyle et al. reported the temperature range for growth of *C. fetus* subsp. *jejuni* as 90 to 113°F. The optimum range for growth seems to be 107.6 to 113°F.

Ordinary cooking, which destroys *Salmonella* spp., also destroys *Campylobacter* spp. Doyle (1984) reported that heating meat to 140°F and holding it at this temperature is sufficient to destroy any *Campylobacter* present.

pH. The pH range for growth is 5.0-8.0.

Atmosphere. The organism is microaerophilic and requires an atmosphere of reduced oxygen for growth. Optimal growth conditions require 5 to 10% oxygen and 2 to 10% carbon dioxide. Because of its sensitivity to air and the relatively high temperature required for growth, growth of *C. jejuni* in foods is unlikely under ordinary conditions of food handling.

Salt tolerance. At 107.6°F, *C. jejuni* will grow in 1.5% table salt (sodium chloride, NaCl) and 0.5% NaCl, but not in 2% NaCl.

Survival. The organism does not grow in milk, but will survive 22 days at 39.2°F. If milk is held at 77°F, destruction of the microorganism occurs within 3 days. *Campylobacter jejuni* can survive on raw chicken held at - 4°F for more than 64 days (Oosterom et al., 1983).

Infective Dose

A pathogenic dose is usually given as ranging from 10⁶ to as few as 400 to 500 organisms (Walker et al., 1986; FDA, 1993). Host susceptibility seems to dictate infectious dose. The pathogenic mechanisms of *C. jejuni* are still not completely understood. It does produce a heat-labile toxin that may cause diarrhea. It may also be an invasive organism (FDA, 1993)

Symptoms

The symptoms of illness caused by *C. jejuni* are similar to those caused by other enteric pathogens such as *Salmonella* spp., *Shigella* spp., and *Escherichia coli*. Stool cultures can provide positive identification.

Symptoms may be mild to quite severe and appear 2 to 5 days after ingestion of contaminated food or water. In severe cases, ingestion of *C. jejuni* produces severe, even bloody, diarrhea with fever, nausea, and severe abdominal pain. The occult blood may continue for 2 to 3 days after the symptoms are first observed.

Interestingly, children seem less seriously affected than adults who may appear to have ulcerative colitis. The illness may linger 1 to 2 weeks in all ages. Occasionally there may be a relapse characterized by a recurrence of abdominal pain and mild to severe gastroenteritis and bloody diarrhea, which may last for several weeks. The illness can also cause a reactive form of arthritis.

Outbreak Example. The following example appeared in MMWR 35(19):311-312, 1986.

Campylobacter Associated with Raw Milk Provided on a Dairy Tour - California. On October 3, 1985, students and teachers from northern California and some of their family members made a field trip to a San Joaquin County dairy. Of the 50 attendees from whom information was available, 23 (46%) became ill with *Campylobacter jejuni* infection.

Twenty-three (59%) of the 39 attendees who drank raw milk, and none of the 11 who did not drink it, became ill. Included among the cases was an infant who had been almost exclusively breast-fed and became ill after drinking a bottle filled with raw milk at the dairy. In addition, secondary cases occurred in 2 women who had not visited the dairy but who tended an infant who drank raw milk and developed *Campylobacter* gastroenteritis. Stool cultures from 1 asymptomatic and 8 ill

persons grew *C. jejuni*. Neither the cows nor the milk were cultured.

Of the 23 ill field-trip attendants, 96% reported diarrhea; 35%, abdominal cramps; 35%, fever; 26%, vomiting; and 22%, bloody diarrhea. Incubation periods ranged from 1 day to 10 days, but were 3 or 4 days in most cases. Symptoms most commonly lasted 5 days.

Numerous outbreaks of enteric diseases have occurred among school children given raw milk while on field trips to dairies in the United States. As a result, in January 1985, the U.S. Food and Drug Administration (FDA) issued a "milk advisory" to all state school officers recommending that children not be permitted to sample raw milk on such visits.

Healthy lactating cows can carry *C. jejuni* in the intestinal tract, providing an extrinsic source of contamination. Fourteen (61%) of 23 *Campylobacter* outbreaks reported to CDC from 1980 to 1982 were traced to consumption of raw milk. Since culture of diarrheal stools for *C. jejuni* became common, many raw milk-associated *Campylobacter* outbreaks involving thousands of cases have been reported.

Milk is an excellent vehicle for infection, because its fat content protects pathogens from gastric acid and because, being fluid, it has a relatively short gastric transit time. Present technology cannot produce raw milk that can be assured to be free of pathogens. Milk must be pasteurized to ensure the destruction of *Campylobacter jejuni*. In Scotland, the incidence of illness due to *C. jejuni* has decreased markedly since 1983 when the sale of raw milk was banned.

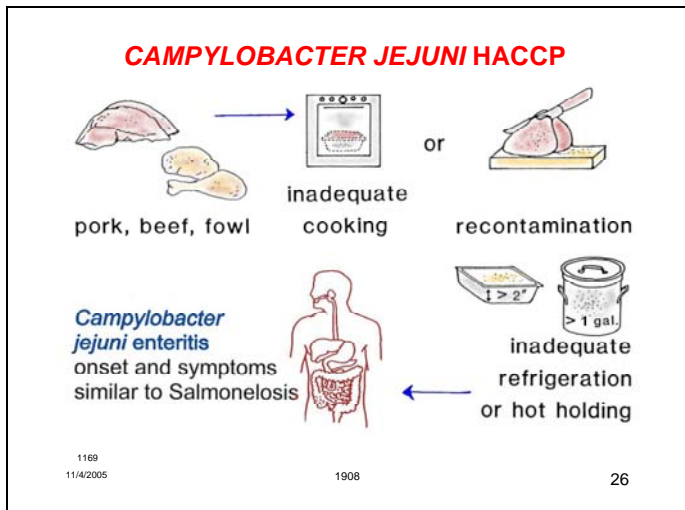
Campylobacter jejuni is present in 30% of raw pork, 80-100% of raw poultry, and 10% of all raw, unpasteurized milk and dairy products. It is found on raw vegetables fertilized with animal or human fecal material.

Incidence

Bennett et al., 1987 estimated an annual incidence of 2,100,000 cases of *Campylobacter* illness in the U.S. resulting in 2,100 deaths. The annual incidence as estimated by the FDA (1993) is 2 million to 4 million cases a year. Roberts and van Ravenswaay, 1989 estimated the annual cost of campylobacteriosis at about 1 billion dollars.

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Campylobacter jejuni - Process Hazard Analysis and Critical Controls

Transmission

Infected humans and animals excrete the organisms in their feces. High numbers of this pathogen (10^6 per gram) are passed in the diarrheal stools of infected individuals. The transmission to humans may be by direct contact with infected people, animals or poultry; through contaminated carcasses and contaminated food and water. This means that cross-contamination on a cutting board or from a contaminated knife can create an instant hazard in another food that is prepared on that same cutting board or knife if that food is not heated sufficiently.

Foods most often implicated are poultry products, unpasteurized milk, meat and eggs, and uncooked foods such as salads and sandwiches that have been contaminated by meat or poultry products, by an infected food handler, or by untreated sewage.

Poultry is a common source of *Campylobacter jejuni*. Heavily infected flocks of chickens can contaminate an entire slaughtering operation. The microorganism can be isolated from the scalding water, pickers, and chilling tanks. Contaminated raw products may then cross-contaminate utensils, work surfaces, and cutting boards in any area where food is prepared.

Control

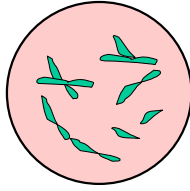
Methods to control the transmission of this microorganism include:

1. Good personal hygiene by food handlers. This includes the frequent use of acceptable methods of hand washing.
2. Sanitary food handling, which includes preparation of raw foods, particularly meat and poultry in separate areas from the preparation of cold or pre-cooked food products.
3. Adequate pasteurization of meat and poultry to ensure the destruction of the microorganism.
4. Avoidance of consumption of raw milk and dairy products.

While microbiological criteria may not be applicable, surveys to ascertain the incidence of this organism in the general food supply should be encouraged. Investigations of foodborne gastroenteritis outbreaks should include examination of suspect food for the presence of *C. jejuni*. Methods for detecting *C. jejuni* in foods are now available.

CHARACTERISTICS OF *ESCHERICHIA COLI*

- Grows with and without air.
- Grows between 45°F and 114°F.
- Survives freezing temperatures.
- Many strains are harmless.
- Source of pathogenic strains is infected animals and people.
- Found in raw milk, raw meat, non-chlorinated water, contaminated fruits and vegetables.
- Vegetative cells multiply and produce toxins in intestinal tract to cause illness.
- Can be low infective dose (*E. coli* O157:H7) = 10 to 100 cells in a portion of food for children.
- Vegetative cells killed by cooking / pasteurization.



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Escherichia coli - Characteristics

Bacterial Characteristics

Escherichia coli is a gram negative, non-spore forming rod. It may or may not be mobile. (Some rods are flagellated and some are not.) The organism is a facultative anaerobe and ferments simple sugars such as glucose to form lactic, acetic, and formic acids.

Growth Conditions

The optimal conditions for growth are a temperature of 98.6°F, with a range of 45 to 114°F. The following table indicates the generation times for *E. coli* O157:H7.

GROWTH OF *E. COLI* O157:H7 IN TRYPTICASE SOY BROTH AT DIFFERENT TEMPERATURES*

Temperature °F	Generation Time (minutes)
36	no growth
77	87.6
86	34.8
99	30.0
104	38.0
111	65.0
113	72.6
114	no growth

*Adapted from Doyle and Schoeni (1984)

The optimum pH for growth is 6.0 to 8.0. However, growth can occur as low as pH 4.3 and as high as 9 to 10 pH (Banwart, 1983; Mitscherlich and Marth, 1984).

Escherichia coli O157:H7 can survive in ground beef at -4°F for several months without change in numbers (Doyle and Schoeni, 1984).

Source

Escherichia coli is a common inhabitant of the intestinal tract of man and warm-blooded animals. Most strains of *E. coli* are harmless and are a part of the normal intestinal microflora. These strains serve a useful function in the body by suppressing the growth of harmful bacteria and by synthesizing appreciable amounts of vitamins.

However, within the species, there are 4 strains or categories that cause diarrheal illnesses or disease. These 4 categories are:

enteropathogenic *E. coli*, enteroinvasive *E. coli*, enterotoxigenic *E. coli* and enterohemorrhagic *E. coli*.

Enteropathogenic *E. coli* causes severe diarrhea in infants that can last for over 2 weeks and results in death if dehydration is severe. In adults, the illness is characterized by severe diarrhea, nausea, vomiting, abdominal cramps, headache, fever, and chills. The time for onset of the illness is 17 to 72 hours; the duration of the illness is 6 hours to 3 days. This strain has caused illness to develop in people when it was transmitted in fecally contaminated water and a coffee substitute.

Enteroinvasive *E. coli* is similar to shigellosis and is caused by bacterial penetration and destruction of intestinal mucosa. Symptoms include: chills, fever, headache, muscle pain, abdominal cramps, and profuse diarrhea. The illness occurs 8 to 24 hours after ingestion of food or water containing this organism. The ingestion of a large number of cells (10^4 to 10^5 cells) is required to cause the illness. An outbreak of this type occurred in the United States in 1981, which was traced to imported French Brie and Camembert cheese. Bacterial counts of the cheese revealed that there were 10^5 to 10^7 *E. coli*/gram. These strains are biochemically and culturally different from other strains of *E. coli*.

Enterotoxigenic *E. coli* include strains that produce enterotoxins when the organisms multiply in the intestine. These strains are commonly responsible for "traveler's diarrhea". They have been responsible for illness in India, in U.S. soldiers in Vietnam, and in travelers in Mexico. This is a problem for travelers from developed countries with good hygiene who visit countries with poor hygiene standards.

The illness is characterized by severe diarrhea, which may lead to dehydration. The diarrhea may last up to 19 days. Usually there is no fever. The onset of the illness can occur 8 to 44 hours after ingestion. Infective dose, as determined by a human study, is 10^8 to 10^{10} microorganisms.

In 1974, more than 2,000 staff members and visitors at Crater Lake National Park in Oregon developed gastrointestinal illness due to this strain of *E. coli*. The source of the microorganism was traced to the park's water supply, which had been contaminated with raw sewage. In 1980, more than 400 persons became ill with gastroenteritis after eating at a Mexican style restaurant in Wisconsin. Enterotoxigenic *E. coli* was identified as the microorganism responsible for this outbreak. A food handler, who had a diarrheal illness during the 2-week period before the outbreak was believed to be the source of the infection.

Enterohemorrhagic *E. coli* (*E. coli* O157:H7) is characterized by severe abdominal cramps usually, but not always, followed by bloody diarrhea (hemorrhagic colitis). Some individuals exhibit only watery diarrhea. Vomiting may occur but there is usually little or no fever. The incubation period is usually about 3 to 9 days.

This microorganism can also cause hemolytic uremic syndrome in children. This is the leading cause of kidney failure in children, which often requires dialysis and may ultimately lead to death.

Other manifestations of illness due to this microorganism include a central nervous system involvement in which patients develop blood clots in the brain and death frequently results.

In 1982, two outbreaks of hemorrhagic colitis occurred, one in Oregon and one in Michigan. Both outbreaks were traced to fast food restaurants of the same chain. Infected individuals had eaten hamburgers that were contaminated. Frozen ground beef patties had not been heated sufficiently to inactivate *E. coli* O157:H7.

In the fall of 1988, an incident occurred at a junior high school in Minnesota that led to illness in 30 students. Four were hospitalized. There were no fatalities. Frozen, partially cooked beef patties were incriminated in this incident. The patties had not been heated sufficiently by the processor to inactivate *E. coli* in the center. The beef patties were reheated before they were served to students, but some, again, were not reheated adequately to inactivate *E. coli* O157:H7.

From November 15, 1992 through February 28, 1993 more than 500 laboratory confirmed infections with *E. coli* O157:H7 and four associated deaths occurred in four states (Washington, Idaho, California, and Nevada). Many young children were involved. The outbreaks were traced to a fast food chain and restaurants in these states serving regular hamburgers and jumbo hamburgers. A meat traceback by the Centers for Disease Control team identified five slaughtering plants in the United States and one in Canada as the likely sources of carcasses used in the contaminated lots of meat. The animals slaughtered in these slaughtering operations were traced to cattle auctions in six western states.

Additional cases of *E. coli* O157:H7 resulted through transmission in families and in child day care settings. As a result of this outbreak, the FDA recommended that all ground meat products be cooked to 155°F for 15 seconds (FDA Food Code). The color of cooked meat is not an indication of its safety.

Other outbreaks of *E. coli* O157:H7 have been found to be associated with cider, apple juice, lettuce and alfalfa sprouts. These incidents point out that food items cannot be presumed safe unless processors or producers utilize procedures for producing safe products.

Infective Dose

Dupont et al. (1971) determined on the basis of a human study that ingestion of 10^6 to 10^8 cells of some pathogenic strains of *E. coli* were needed to cause diarrheal illness in a healthy individual.

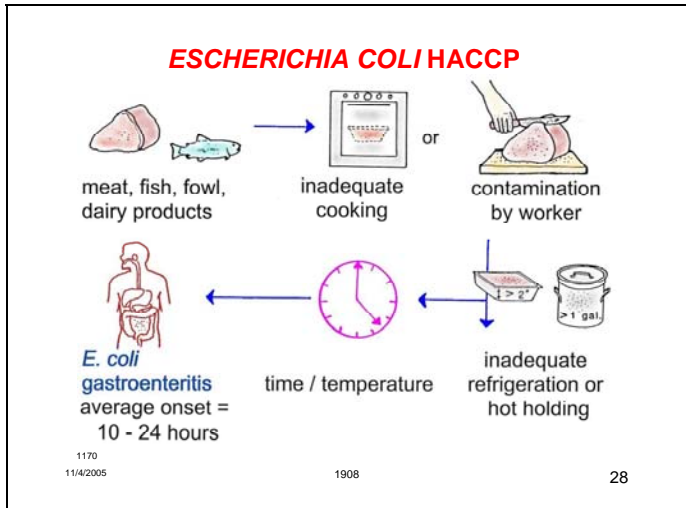
According to the FDA (1993) the infectious dose for *E. coli* O157:H7 is unknown. However, from a compilation of outbreak data, it may be as low as 10 organisms. This data indicates it takes a low number of microorganisms to cause illness in young children, the elderly and immune-compromised people.

Incidence

There is an estimated annual incidence of over 200,000 cases of enteric (intestinal) *E. coli* in the United States each year, resulting in 400 fatalities (Bennett, 1987).

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Escherichia coli - Process Hazard Analysis and Critical Controls

Transmission

Raw foods, particularly those of animal origin, are frequently contaminated with *Escherichia coli*. Raw milk can contain *Escherichia coli*. People are also carriers of this microorganism and can transmit the microorganism to food products through fecal contamination as a result of inadequate hand washing.

Escherichia coli is found on fish and shellfish taken from sewage-polluted waters. If a polluted water supply is used to water fresh fruits and vegetables, these foods too will become contaminated.

Carcasses are often contaminated with fecal material of infected animals or from other contaminated carcasses or equipment. It has been noted recently that young cattle are more likely to be a source of this microorganism than older cattle.

Prepared foods can become contaminated with *Escherichia coli* from equipment that has not been cleaned and sanitized after it was used to prepare raw food products and from infected food handlers.

Control

Control of enteropathogenic types of *Escherichia coli* in food can be attained by:

1. Mandating proper hand washing procedures for food handlers.
2. Purchasing food, particularly meat and poultry, from suppliers who certify the safety or microbiological quality of their products.

The FDA developed destruction standards for *Escherichia coli* O157:H7 in ground beef in 1993 based on the data of Line et al., (1991). In the following table, the D values at 125°F, 135°F, and 145°F come from this research article. The FDA used these data to extrapolate mathematically to the destruction values at 140°F, 145°F, 150°F, and 155°F. The z-value for both of these sets of data is approximately 8.3°F.

Using these data from these sources, the table indicates times needed to destroy 90% (1 decimal reduction or 1 D-value) of *Escherichia coli* O157:H7 in ground beef and the time to destroy 99.999% (5 decimal reductions or 5 D-value).

THERMAL INACTIVATION TIMES OF ESCHERICHIA COLI O157:H7 IN GROUND BEEF

Temperature (°F)	5 D-values
140	8.7 minutes
145	2.7 minutes
150	52 seconds
155	15 seconds

If food products are not purchased from suppliers who certify safety:

1. Food, particularly ground beef, must be cooked or heated according to thermal inactivation standards given by Table 1, or heated to 155°F for 15 seconds as recommended by the FDA. (This is a 5D *Salmonella* kill.)
2. Raw food preparation areas should be separated from cooked food preparation areas and/or good cleaning and sanitizing methods must be used to ensure that equipment and surfaces do not contaminate other products.

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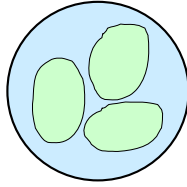
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CHARACTERISTICS OF *LISTERIA MONOCYTOGENES*

- Grows with and without air.
- Grows between 29.3°F and 113°F.
- Found in plant matter and soil, raw milk, raw meat, contaminated prepared foods.
- Source of contamination is infected animals and people, inadequately pasteurized food, floor drains.
- Vegetative cells multiply in intestinal tract to cause illness (severe for immune-compromised, elderly, infants, and pregnant women).
- Low infective dose = 100 to 1,000 cells in a portion of food for immune-compromised people.
- Vegetative cells killed by cooking / pasteurization.



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Listeria monocytogenes - Characteristics

Listeria monocytogenes is a gram-positive, short rod that is motile at 6 to 77°F. It is a facultative (grows with and without air), non-spore forming pathogenic bacteria.

Source

Listeria monocytogenes is commonly found in the environment and has been isolated from both cultivated and uncultivated soil. It is present in vegetables and plant matter, especially in decaying plant material. It has been found in the intestinal flora of humans, animals, fish, insects, birds and poultry. It has been recovered from both raw and treated sewage. Infected cows and sheep excrete *L. monocytogenes* in their feces and milk.

Growth

Listeria monocytogenes grows from 29.3 to 113°F and can increase in number at refrigeration temperatures (Hudson et al., 1994; Grau and Vanderline, 1990). The population will double in number in 1.5 days at 39.2°F [Rosenow et al., 1987]. The following table indicates the predicted generation times for *L. monocytogenes* in foods.

PREDICTED GENERATION TIMES FOR 1 MULTIPLICATION OF *LISTERIA MONOCYTOGENES* IN FOODS*

Temperature °F	Time
32	16 days
35	4 days
41	1.2 days
45	16.7 hours
50	9.8 hours
55	6.0 hours
60	4.5 hours
65	3.4 hours
70	2.6 hours
75	2.1 hours
80	1.7 hours
85	1.4 hours
90 to 100	1 hour

* Adapted from data of Snyder, O.P. (1998).

Foods with a pH of 5.0 to 9.0 will support its growth. The organism has been found to grow in silage as low as pH 3.6. However, the growth of this microorganism is extremely slow at

a pH less than 4.5. *L. monocytogenes* can grow in the presence of sodium chloride at levels of 5% or greater.

The organism can survive repeated freezing and thawing. Pasteurization should control *L. monocytogenes*, if the vegetative cell population is not greater than 10³/g. Any product that is not heated or pasteurized sufficiently before being chilled or frozen and consumed is a potential carrier of *L. monocytogenes*.

Symptoms

In adults there is a sudden onset of "flu-like" symptoms that include: fever, chills, headache, backache, and sometimes abdominal pain and diarrhea. These symptoms may precede more serious complications, which include septicemia, meningitis, encephalitis, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortions during the second or third trimester, or in stillbirths. Pregnant women usually suffer painful, short-term effects but their unborn fetuses are at greatest risk. Surviving infants often contract meningitis or other complications. Newborn infants, whose mothers had listeriosis, have respiratory problems, refusal to swallow, vomiting, and nodules in the throat or on the back.

The elderly and adults with underlying health problems (compromised immune systems) often die from meningitis or other complications that result from listeriosis.

Listeriosis is only positively identified by culturing the organism from blood, cerebrospinal fluid, or stool samples.

Infective Dose

The infective dose is not known at this time, but is probably less than 1,000 total microorganisms in susceptible persons (FDA, 1993).

Incidence

Healthy children and adults are usually not made ill by these microorganisms. However, an outbreak of listeriosis in Switzerland involving cheese suggests that healthy uncompromised individuals may develop the disease if the food product is heavily contaminated. Taking antacids may even make seemingly healthy individuals more susceptible (FDA, 1993).

Listeria monocytogenes is a great risk to the elderly, infants, and pregnant women and their unborn children. Immune-compromised people with cancer, AIDS, cirrhosis, and kidney disease, or those who have undergone organ transplant are also at risk for contracting listeriosis.

In 1981, 41 cases of listeriosis were reported in Nova Scotia. There were 34 cases of perinatal listeriosis in this outbreak. As a result of this illness in these pregnant women, there were 5 spontaneous abortions, 4 stillbirths, 23 cases of live births of seriously ill infants and only 2 live births of well infants. The outbreak was due to the consumption of coleslaw made from cabbage fertilized with sheep manure. It was determined that the sheep had died previously of listeriosis.

In 1983, 49 individuals in Massachusetts acquired listeriosis and 14 people died. This outbreak was traced to "pasteurized" milk. The incident was thought to be due to raw milk, which was highly contaminated, and inadequately pasteurized, or contaminated dairy processing equipment.

In 1985, 86 cases of *L. monocytogenes* infection were identified in California. More than 1/2 of the patients were pregnant women. Forty-two of the infants delivered from these women had listeriosis within 24 hours of birth. The source of the infection was a soft Mexican cheese manufactured at a plant in southern California.

In 1988, a woman with cancer was hospitalized in Oklahoma with sepsis caused by *L. monocytogenes*. *L. monocytogenes* was isolated from an open package of turkey franks from the patient's refrigerator. The patient had eaten one turkey frank daily, heated in the microwave oven. *Listeria monocytogenes* was isolated from packages of turkey franks at a local retail store and was traced to a processing plant. Cultures of other foods in the patients' refrigerator were also positive for *Listeria monocytogenes*. This finding indicates that other opened packages of food can become cross-contaminated if *L. monocytogenes* is present in the surrounding environment.

In 1989, the estimated annual incidence of this illness in the United States was 25,000, resulting in as many as 1,000 fatalities (Todd, 1989).

In the winter of 1998-1999, an outbreak of listeriosis occurred when it was discovered that processed cold meat products were contaminated with *L. monocytogenes*. Product recalls were extensive. Poor sanitation measures at a meat processing facility is suspected as a contributing factor to this illness outbreak.

Food Analysis

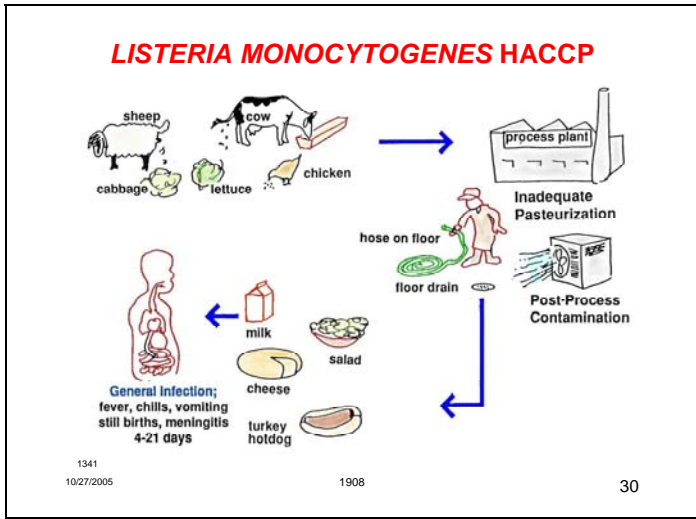
Present methods of analyzing foods for this organism are time consuming and complex. The total time for identification requires 5 to 7 days. Newer methods of analysis are expected to shorten this time.

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Listeria monocytogenes - Process Hazard Analysis and Critical Controls

Transmission

Listeria monocytogenes is an animal pathogen. The first documented human cases of listeriosis traced directly to food occurred in 1981, although human cases had been reported since 1930.

The major problem with this organism is contamination after heating. Outbreaks have been traced to raw products (e.g., cole slaw) as well as post-pasteurized milk products (e.g., ice cream, soft cheese) and cured meat products (frankfurters). When the presence of *L. monocytogenes* is discovered in food products or on surfaces, the population is so large that its elimination is a major challenge.

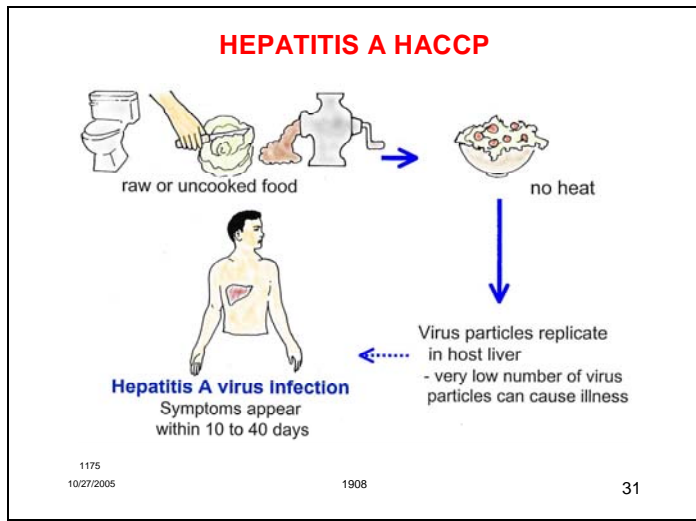
Control

In order to prevent listeriosis:

1. Only pasteurized milk and dairy products should be consumed.
2. Products stored at 41°F or less should be consumed within 7 days after receipt, or according to correct holding times.
3. Refrigerated or frozen products should be reheated until all parts of the food reach 165°F for 15 seconds.
4. People at risk should not consume uncooked vegetables and salads.
5. Food must be heated adequately. The following D values are recommended for the destruction of *L. monocytogenes*.

Temperature °F	Time (1D) (minutes)	Time (4D) (minutes)
130	21.95	87.80
140	2.85	11.40
150	0.37	1.48
160	0.047	0.19

These times and temperatures are based on a strain of *L. monocytogenes* called Scott A. The values were based on the average of heat resistance of this strain in ground beef of high fat content (fat increases heat resistance) and lean ground beef.



1175

Hepatitis A Virus Food Infection

Hepatitis A virus is very small. Purified virus particles are 27 nanometers in diameter and possess single-stranded RNA (nucleic acid). Hepatitis A virus is the cause of the highest number of reported viral food-associated illnesses. The virus is found in feces, urine, and blood of infected persons. The illness caused by hepatitis A virus is also known as infectious hepatitis and is completely unrelated to illness caused by hepatitis B virus (serum hepatitis), which is usually only transmitted by blood-to-blood contact.

Hepatitis A virus does not grow on food. It replicates itself in the liver of its human host and is passed in the feces, urine, and blood of infected individuals (who may be without symptoms). It is also found in waters containing raw sewage and in seafood taken from these polluted waters.

Hepatitis A Virus Transmission

Hepatitis A viral foodborne illness is most often associated with raw foods. It can also be associated with prepared foods that become contaminated during further processing or serving.

Infectious hepatitis can be caused by relatively few virus particles, probably as few as 5 to 10 per gram of food. The disease is transmitted by an infected food handler or food harvester who is either ill at the time of transmission, or who is a carrier. This person excretes the virus in the feces and urine. When fingertips and fingernails are not washed carefully and adequately, the virus is spread to food.

Viruses are also transmitted through contaminated water. Shellfish (oysters, clams, and mussels) taken from waters contaminated with raw sewage are a source of hepatitis a virus.

Symptoms

The onset of symptoms is usually abrupt and is characterized by fatigue, fever / chills, loss of appetite, nausea, vomiting, pain in the liver area, abdominal pain, jaundice, dark urine, and light-colored stools. Jaundice occurs because the virus invades the liver and affects its function. As a result, the pallor or skin tone of infected individuals takes on a yellow color.

The illness may be mild in some cases and only last from 1 to 2 weeks. The most important factor affecting the severity of the disease is age. Children less than one year old rarely show

clinical signs of illness. This means that parents and child-care workers handling soiled diapers can catch or transmit the disease without knowing they have been exposed. Clinical manifestations of hepatitis A often pass unrecognized in children younger than two years of age. In most younger people, there is complete recovery with no long-term effects. A clearly recognizable illness due to hepatitis A develops in the majority of infected older children and adults. In some cases, the illness can be severely disabling, can last for several months, and may cause some permanent liver damage. The severity of the illness is usually greatest in elderly people and may cause death. An estimated 100 deaths occur in the U.S. each year from hepatitis A.

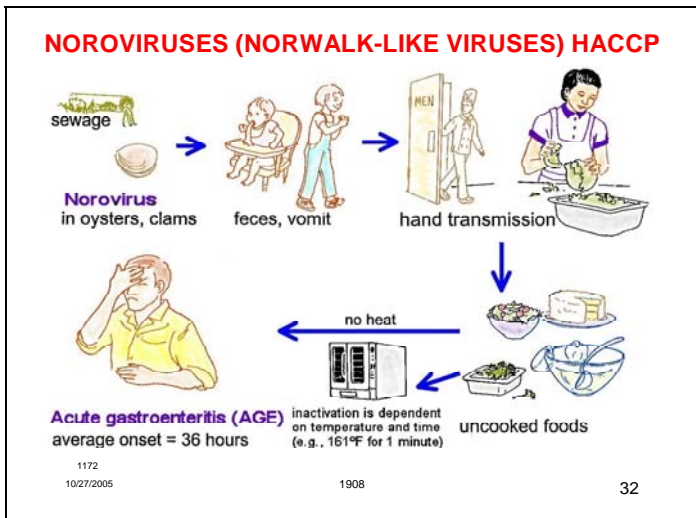
Hepatitis A Virus Control

Raw fruits and vegetables must be double washed in a sanitized sink. The water dilutes and removes the viruses on the surface to a level low enough that the hazard is controlled.

All employees in foodservice and food production must practice good hygiene. Employees must use the double hand wash procedure that uses a fingernail brush and soap and to scrub hands and fingernails after using the toilet. This virus can be shed in the feces and urine days or weeks before a person feels ill and goes to the doctor.

Food preparers and servers must use only clean, sanitized equipment and utensils to mix and serve food.

Viruses, although incapable of multiplying in food, may remain viable in food for weeks and in frozen food for months. Food containing hepatitis A virus must be heated to temperature above 180°F.



1172

Noroviruses Food Infection

Noroviruses (Norwalk-like viruses) are small, round-structured viruses. A virus of this type was first isolated in 1972 in Norwalk, Ohio. Since that time, these viruses have been identified as a cause of gastroenteritis in many countries throughout the world. The amount of norovirus needed to cause illness is unknown, but probably very low, perhaps 1 to 10 virus particles. The annual estimated incidence of illnesses due to noroviruses is over 9,000,000 cases. These viruses are thought to be responsible for more foodborne illnesses in the U.S. than any other bacterial, parasitic, or viral agents.

Transmission

Noroviruses do not multiply in food but may be acquired through direct contact with an ill person and/or carried by air, water, or uncooked food to a human in which the virus will multiply. Humans are the source of these viruses by way of fecal-oral transmission. Children and staff members can spread these viruses in day-care centers through changing of diapers and inadequate hand washing after touching fecal material and other body fluids.

These viruses are recognized as an important cause of waterborne illness. Under naturally occurring conditions in a contaminated water supply, routine chlorination alone will not inactivate these viruses. If drinking or recreational water is suspected as being an outbreak source, high-level chlorination may be required for adequate disinfection; however, even this method may be insufficient in some cases.

Noroviruses have been transmitted on oysters, cold precooked ham slices, icing, salads, and water. Reported inactivation of suspended caliciviruses used to simulate noroviruses has been shown to be temperature and time dependent in the range from 0 to 100°C (32 to 212°F). According to Duizer et al. (2004), a 1,000-to-1 reduction was noted at 71.3°C (160.3°F) for 1 minute.

Virus transmission is a concern in products that receive no heat processing after contamination (e.g., salads, sandwiches, any cold foods). Noroviruses can survive refrigeration and even freezing.

Infection Characteristics

The illness causes extreme discomfort but is rarely fatal. Noroviruses are very infective. Symptoms include nausea, abdominal pain, anorexia, headache, and sometimes fever. The nausea produces much vomiting in children, but in adults tends to produce diarrhea. Symptoms are due to infection of the intestinal lining. Usually the symptoms appear within 24 to 48 hours of ingestion of the virus but can occur within 12 hours of exposure. It can last from 24 to 60 hours. Once an infected person has recovered, the disease may be passed to others for up to 2 weeks after recovery. This way, the illness can spread easily through schools, camps, and families.

All individuals who ingest the virus and who have not recently (within 24 hours) had an infection with the same or related strain, are susceptible to infection and can develop symptoms of gastroenteritis (FDA, 1993).

The annual estimated incidence of illnesses due to noroviruses is over 9 million cases (Mead et al., 1999). These viruses are thought to be responsible for more foodborne illnesses in the U.S. than any other bacterial, parasitic, or viral agents.

Outbreaks

1. A sick baker returning from a bathroom contaminated an 80-quart bowl of icing with hands that did not appear to be dirty. The icing made over 5,000 people ill when it was used for wedding cakes and other items sold by the bakery.
2. Sick salad preparation worker did not wash her hands properly after using the bathroom. As a result, the salads were contaminated with the virus and over 3,000 customers became ill from the contaminated salads, which were sold and consumed over a 3-day period.
3. An elementary school student vomited on a floor in an open classroom. In about 30 hours, 60% of the other students were ill from the airborne transmission of a norovirus.

From January 1996 through November 2000, there were 348 outbreaks of norovirus gastroenteritis reported to the Centers for Disease Control.

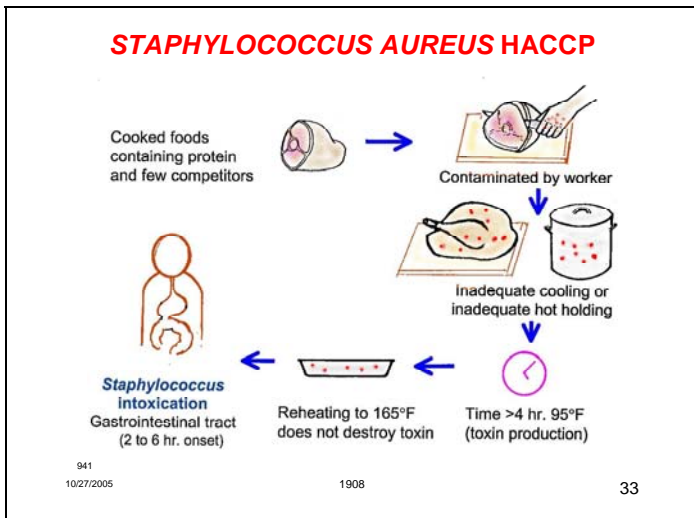
Control

Noroviruses are usually transferred to food by people's hands and contaminated water. Therefore, in order to control outbreaks of this illness:

1. Foodservice workers must use good personal hygiene and practice the two-step hand washing procedure, using a fingernail brush to scrub hands and fingernails after using the toilet.
2. Foods that are to be served uncooked (e.g., raw vegetables and fruits) must be washed thoroughly in flowing water, using a safe water supply.
3. Suppliers of seafood should certify that their seafood products were obtained from safe waters.
4. To ensure the destruction of the virus in seafood products and other heated food products, these products should be heated to about 161°F for 1 minute.
5. Clean, sanitized equipment should be used to mix, serve, and store food.

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Staphylococcus aureus Food Intoxication

Staphylococcus aureus is common in man. These bacteria are characterized by a group of cocci combined in grape-like clusters. It can be found in the noses of 30 to 50 percent of healthy people and on the hands of 20% of healthy people. *Staphylococcus aureus* is also commonly found in the throat, on hair, in feces, and on skin of people and animals. It grows in the presence or absence of air. Thus, it can grow on the surface of many food products. It requires protein to grow and will grow well in meat, milk, poultry, fish, eggs, and custards. It is very tolerant of salt and sugar and can multiply in foods with high salt and sugar concentrations, such as hams and cream-filled pastry products, which are not suitable for the growth (multiplication) of many other bacteria. It does not compete well with spoilage bacteria. Hence, it is usually a problem in cooked, pasteurized food where other competitive microorganisms have been destroyed.

The viable cells of *S. aureus* require about twice as much time for inactivation as *Salmonella* spp. at specified temperatures. However, it is not the ingestion of the viable *S. aureus* cells that causes the illness. Individuals can consume 1,000 *S. aureus* cells per gram in food without becoming ill. The illness is due to ingestion of foods containing the toxin produced when viable cells multiply within or on food products, usually over 1,000,000 per gram, and produce enough toxin to make normally healthy persons ill. The toxin can survive hours of boiling.

Because *S. aureus* and other some other types of pathogenic bacteria produce toxin that is not destroyed by the normal reheating temperature of 165°F, it is not possible to guarantee that reheated food is safe at 165°F. To assure safety of food, particularly cooked food for which there is no spoilage bacterial competition, it must be handled in such a way that there is no chance of toxin production. The FDA Food Code recommends cooling food from 135 to 70°F in 2 hours and within a total of 6 hours from 135 to 41°F, or serving within 4 hours if the food is at warm temperatures. [USDA Guidelines recommend continuously cooling food, within 90 minutes after cooking, from 120°F to 55°F within 6 hours, followed by further cooling to 40°F (no time limit) before boxing.]

Staphylococcus aureus Transmission

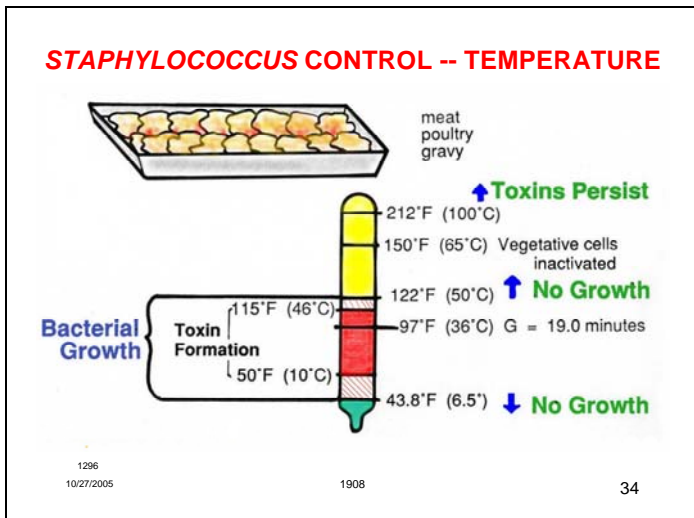
Staphylococcus aureus is often present in products of animal origin. It is a normal skin contaminant of animals, and when they are slaughtered, it is common to find 10 to 100 bacteria per gram in meat. At this level the bacterial count is too low to produce enough toxin to make persons ill.

Other major sources are infected cuts on employees' hands. In this case the food usually becomes contaminated during mixing, slicing, or poor handling procedures. In these circumstances, high numbers of vegetative cells introduced into food can produce enough toxin in 6 to 9 hours to cause illness.

If food contaminated with *S. aureus* is allowed to remain at room temperature for long periods of time (e.g., 9 hours at 75°F), held in warming tables or cabinets at 80 to 110°F, or stored in warm, large containers that take many hours to cool in refrigerators (e.g., making salads with warm ingredients), the viable cells of *S. aureus* will multiply and produce enough toxin to cause foodborne illness when the food is eaten.

Illness Characteristics

The ingestion of food containing the toxin can cause people to become ill within 2 to 4 hours. Symptoms of the illness are nausea, vomiting, abdominal cramps, and diarrhea. The symptoms can be violent for a short period of time. *S. aureus* intoxication rarely causes death.



1296

Staphylococcus aureus Control

Most meats, poultry, and many other foods have 10 to 100 *Staphylococcus aureus* per gram. The *S. aureus* must not be allowed to multiply to greater than 1,000,000 per gram because that amount can produce enough toxin to make the average person ill. At low levels (less than 1,000 per gram) *S. aureus* is not a threat. The vegetative cells of *S. aureus* are slightly more difficult to inactivate than those of *Salmonella* spp. in cooking, so there can easily be low levels of *S. aureus* in foodservice items.

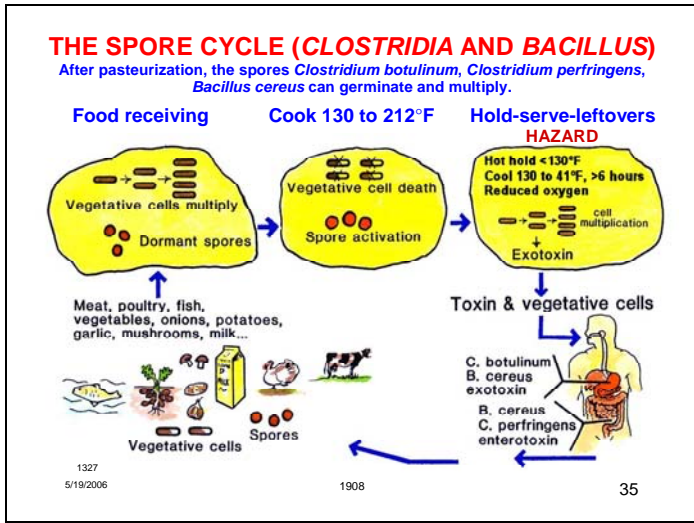
Multiplication of this microorganism is possible during cooling when contaminated ingredients are mixed and the food does not cool quickly enough. Manipulation of temperature is an effective method of control. There is no multiplication of *S. aureus* below 43°F or above 122°F. Most rapid multiplication occurs at 97°F (e.g., one generation of growth in milk every 19 minutes). A key hazard control is the fact that toxin is formed between 50 and 115°F.

To inactivate (kill) vegetative cells of *S. aureus*, cook food to above 140°F according to the *Salmonella* spp. time-temperature kill standards. To control the growth of this microorganism, cool food according to the FDA Food Code or the USDA Guidelines.

The key to controlling the growth of *S. aureus* in foods, particularly salads, is the fact that it does not produce a toxin below 50°F. For example, when salads must be mixed by hand to maintain the integrity of the ingredients, the hands will introduce a few *S. aureus* (probably less than 10 per gram), but this is not a problem until the *S. aureus* multiplies. When each salad ingredient is cooled individually before mixing, the ingredients can be mixed safely if the temperature of the mixture is kept below 50°F. A good professional standard to use in preparing salads is to cool all ingredients to 41°F and then mix all cold ingredients so that the salad mixture stays below 50°F. Never cut up food in the range of 80 to 115°F, mix it, fill up a deep container such as a 5-gallon plastic pail, and then try to cool the food. It will take days. This makes it easy for *S. aureus*, added to the food from the hands, to produce a toxin in the salad during cooling. The higher the temperature, the less the acid in the salad dressing acts as a hurdle to prevent the growth of *S. aureus*.

Since it can multiply as quickly as once every 19 minutes, food can become hazardous in just a few hours at optimum growth temperatures.

The control of foodborne illness caused by *S. aureus* should include use of good sanitary precautions by foodservice employees (i.e., proper hand washing techniques after touching pimples and infected cuts as well as raw meat products). Foods should be pasteurized correctly and cooled correctly. Salads should be prepared with ingredients at less than 50°F and maintained at temperatures that do not permit production of *S. aureus* toxin.



1327

Spores

Some rod-shaped pathogenic vegetative bacterial cells, such as *Clostridium perfringens* and *Clostridium botulinum*, and *Bacillus cereus*, have the ability to form spores. Spores are formed when environmental conditions around the vegetative cells become poor. When a spore is formed, the reproductive system of vegetative cells is surrounded by a tough shell, and the outer part of the cell falls off. Spores are much more resistant to heat, chemicals, etc. than are vegetative cells. The spore state is a period of no growth, similar to hibernation, in which the cell dries up. Spores are found in soil, water, and the environment. Any food that comes in contact with the soil becomes contaminated with spores. This includes fish (which ingest mud), vegetables, potatoes, rice, flour, milk, eggs, meat, and poultry.

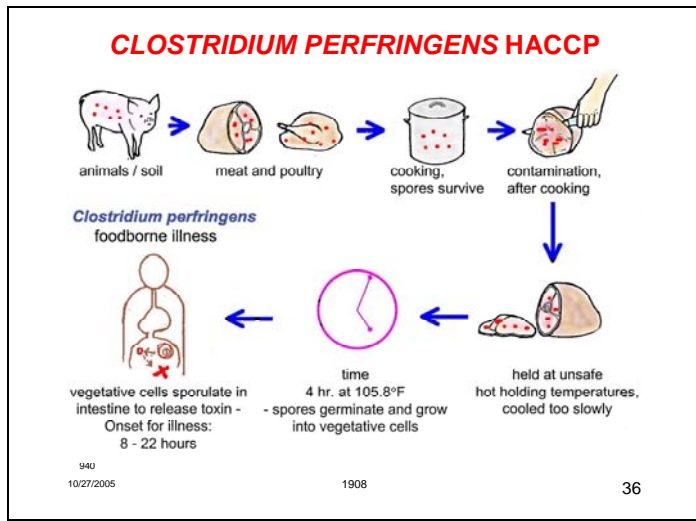
The Spore Cycle

The spore cycle begins when foods such as vegetables and cereals are harvested, and meat and poultry are slaughtered. The cycle continues when the food is received and heated at the foodservice. Raw food at 41°F has vegetative cells and some *Clostridia* and *Bacillus* spores. During initial heating, the vegetative cells of *Clostridia* and *Bacillus* can multiply between 38 to 125°F. Rapid multiplication (less than 1 hour) can take place between 85 to 120°F. Therefore, it is necessary to cook food from 41°F to the safe temperature of 130°F in less than 6 hours. When the food is cooked uniformly to temperatures of 145 to 165°F, sufficient heat has been applied to inactivate the vegetative cells to a safe level. The food becomes pasteurized. The higher the temperature, the faster vegetative cell destruction occurs.

The spores, however, survive. (It takes hours of cooking at 212°F and 3 minutes of the canning temperature of 250°F to assure inactivation of *C. botulinum* spores.) When the product is cooled to below 125°F, the spores can germinate, given more than 4 hours, into vegetative cells that multiply in the food product(s). Holding food at temperatures of 85 to 120°F for as little as 2 hours will allow enough bacterial multiplication of *C. perfringens* to cause foodborne illness. To control spore outgrowth, food must be kept at temperatures greater than 124°F (greater than 150°F for quality and some government regulations). Roast beef should be held 130°F or above, or

cooled from 130 to 41°F in less than 6 hours according to FDA Food Code recommendations. USDA Guidelines recommend continuously cooling food, within 90 minutes after cooking, from 120 to 55°F within 6 hours, followed by further cooling to 40°F (no time limit) before boxing. **Note**, 130°F is a safe holding temperature that is only a few degrees above the multiplication temperature of *C. perfringens*. It is important to note that a small mistake in maintaining holding temperature can cause roast beef to become hazardous in 4 hours if held at 110°F. FDA regulations state that food should be held at 135°F. Some states require 140°F or 150°F. These temperature requirements allow a margin of safety.

During multiplication, *C. botulinum* and one form of *B. cereus* produce toxins that probably will not be inactivated when the food is reheated. People who eat food containing these toxins will become ill and may die. In other foodborne illness cases, it is the large number of vegetative cells of *C. perfringens* and another form of *B. cereus* produced in the food before it is consumed that makes people ill. To complete the cycle, bacteria and spores are passed from the body and returned to the environment through sewage waste to cycle again.



Clostridium perfringens Food Intoxication

Clostridium perfringens is found in soil and dust and in the feces of man and animals. It has two forms: a **spore** form, which survives the cooking process but does not cause illness directly; and a **vegetative cell** form, which the spore becomes when environmental conditions permit growth. The presence of 100,000 vegetative cells per gram in food will cause illness. (The same number of spores will have no effect since the presence of spores of *C. perfringens* in food does not cause the illness.) *Clostridium perfringens* grows only in the absence of air. It can grow in sealed packages of food, in the internal mass of food (e.g., in large pieces of meat or poultry and large containers of casseroles), or in cooked foods as shallow as 1 inch deep.

Clostridium perfringens requires protein for growth. This microorganism is usually present in foods of animal origin such as meat and poultry, and in other protein-containing foods such as gravies, sauces, and soups. It grows well in mashed potatoes, beans, and lima beans. It can survive the curing of meat because the organism is moderately salt tolerant and prefers an anaerobic environment. It can grow in the liquid portion of unsalted butter.

Active vegetative forms of *C. perfringens* can change to dormant, hardy, heat-resistant spores when the environment becomes hostile. The spores can survive indefinitely and are also resistant to dehydration and cold temperatures. Heat at 180°F does not destroy the spores but instead stimulates the spores to germinate and develop into vegetative cells when adequate environmental conditions are met. *Clostridium perfringens* cells multiply very rapidly in cooked food held at room temperature. At 105.8°F, they double every 7 to 8 minutes in ground beef. It takes only a couple of hours for take-out food, held at 90 to 115°F to become hazardous.

Clostridium perfringens Transmission

Clostridium perfringens is transferred to meat and poultry from animal feces during processing, from workers who have not washed their hands after using the toilet, from soil and dust on equipment, and from very small lesions in the animals' intestinal walls. The insides of meat can also become contaminated when it is sliced, chopped, stuck with a dirty fork, or put onto a spit.

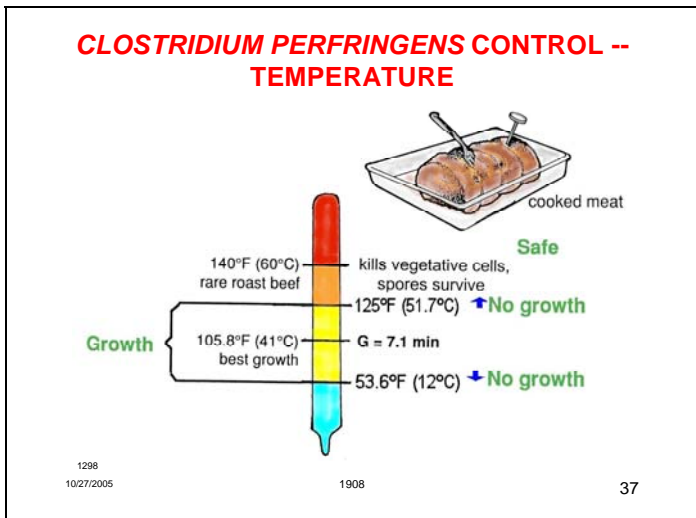
Foods that are allowed to remain between 80 and 120°F, held in warming tables or cabinets at less than 130°F, or cooled in large containers in refrigerators allow the spores to germinate and the vegetative cells to multiply to large numbers. But, in this one case, reheating will make the food safe. Reheating food to above 140°F according to the *Salmonella* time-temperature kill values will destroy the vegetative cells of *C. perfringens* that contaminate the food and make the food safe to eat.

This organism can also multiply during slow heating. Food must be heated from 41 to above 130°F in less than 6 hours to assure the control of this bacteria.

Illness Characteristics

To cause *C. perfringens* foodborne illness, the vegetative cells must multiply to greater than 100,000 per gram in food. After a person eats the food containing the cells of *C. perfringens*, the cells enter the intestine where they release toxin as they sporulate. This release of toxin in the gut causes the person to become ill. The illness (characterized by abdominal cramps and intense, putrid smelling diarrhea) develops in about eight to twenty hours. People recover quickly and are usually able return to work the next day.

Because *C. perfringens* produces no spoilage characteristics when it multiplies in food, its victims often note that the offending food was the "best chili, turkey hamburger, or roast beef they had ever tasted."



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Clostridium perfringens Control

Clostridium perfringens foodborne illness outbreaks result when this microorganism is allowed to multiply to large numbers (more than 100,000) during slow cooking, improper hot holding and inadequate cooling (i.e., the failure to keep hazardous foods contaminated with spores and viable cells of *C. perfringens* out of critical growth range temperatures of 53.6 to 125°F for extended periods of time).

Control Factors

Personnel in food production and foodservice establishments must:

- a. Assume that most raw and cooked meat and poultry products are contaminated with 10 to 100 cells of *C. perfringens* per gram. If these products are kept below 53.6°F, *C. perfringens* will not multiply. The spores will survive cooking and will grow out into vegetative cells when foods are cooled too slowly, or are not held above 130°F.
- b. Use cooking methods that ensure that food products, particularly large roasts, poultry items, and high protein casseroles, pass a center temperature between 41 to 130°F in 6 hours or less. Food should be pasteurized according to *Salmonella* reduction standards. (Remember, this only inactivates the *C. perfringens* vegetative cells, not the spores.)
- c. Use rapid cooling methods for high-protein items. The FDA Food Code recommends cooling food from 135 to 70°F within 2 hours followed by further cooling to 41°F (6 hours or less, total time). USDA Guidelines for cooling recommend continuously cooling food, within 90 minutes after cooking, from 120°F to 55°F within 6 hours, followed by further cooling to 40°F (no time limit) before boxing.

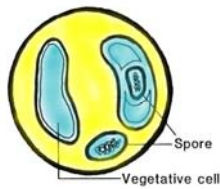
For example, when cooling products:

- 1) Large roasts should be sliced and placed in layers not thicker than 2 inches in depth if cooled in an ordinary refrigerator.
- 2) Stews, soups, casserole items, gravies, and sauces should be placed in pans that are not thicker than 2 inches in depth for cooling in an ordinary refrigerator.
- 3) High-velocity fans must be used within 4 inches of the pans of food cooling in the refrigerator to ensure rapid circulation of air at less than 35°F over the food if cooling in 6 hours is required.

- d. Government regulations for holding food above 135°F provide an extra measure of safety because of the unreliability of thermostats on hot holding equipment.) Customers prefer most hot food to be above 150°F and soup to be at 165°F when they put it in their mouths.
- e. Use good personal hygiene and proper methods of hand washing when handling food products. People carry the organism in their intestines. People can be carriers or shedders.
- f. Use sanitized equipment such as cook's forks, thermometers, and metal spits to penetrate or serve meat, poultry, stews, soups, and casseroles. Unsanitized equipment can inject *C. perfringens* into the center of food products where they will grow well in the anaerobic conditions.

CHARACTERISTICS OF *BACILLUS CEREUS*

- Grows with and without air.
- Grows between 39.2°F and 122°F.
- Source is soil. Found in rice, spices, cereals, milk.
- Forms spores that are resistant to cooking / baking temperatures. Spores grow out as vegetative cells when food cools.
- Two types of illness:
Emetic illness (vomiting) – ½ to 6 hours after ingestion.
Diarrheal illness – 10 to 12 hours after ingestion.
- Heat-resistant toxin(s) – can withstand temperature (250°F) 90 minutes.



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Bacillus cereus - Characteristics

Bacterial Characteristics

Cells of *Bacillus cereus* are large, gram-positive rods that are motile by means of flagella. Cells are aerobic spore formers that are also capable of growing under anaerobic conditions. Spores are formed when conditions for growth of vegetative cells are not present.

Bacillus cereus is responsible for two types of foodborne illness: the emetic (vomiting) illness and diarrheal illness, which are caused by two distinct enterotoxins produced by different strains of this microorganism

Spores of this microorganism are present in many foods from harvest through processing. The organism, normally present in most food, is not a hazard at numbers below 1,000 CFU (colony forming units) per gram. Hazardous levels of this pathogen can develop when food (especially cooked foods in which most competitive microorganisms have been destroyed) is held in the range of 85 to 120°F for a long period of time (e.g., the number of hours typical of cooling a 6 inch, covered pan of cooked rice in an ordinary refrigerator). Under these conditions, the organism can grow to large numbers, releasing toxin during growth in the food and/or in the intestinal tract after the food is consumed.

Recently, psychrotrophic strains of this pathogen were shown to grow at a temperature range of 39.2 to 98.6°F in pasteurized milk, mousses, and cook/chill meals (van Netten et al., 1990).

Source

Bacillus cereus is common in the soil and dust throughout the world. It is frequently isolated in grains, flour, starch, and other cereal products. Prepared foods implicated in the outbreaks of foodborne illness due to *B. cereus* include: mashed potatoes, pasta, macaroni and cheese, feta cheese, stuffing, rice and rice dishes (fried rice), malted milk powder, meat and items made with meat and poultry, soups, instant breakfast products, vanilla puddings and cream sauce, and other products that incorporate cereal products.

This microorganism has been found in a variety of food stuffs. For example, *B. cereus* is a contaminant of many spices. In the

United States, 25% of 175 samples of dry food, distributed nationally, yielded the organism (Kim and Geopfert, 1971).

Growth Conditions

Temperature. Recent research, as reported by van Netten et al. (1990), has shown that psychrotrophic strains of *B. cereus* are capable of growth at 39.2°F. The maximum temperature for vegetative cell growth and spore outgrowth is 122°F (Kramer and Gilbert, 1989). Optimum growth occurs at temperatures of 82 to 95°F (Adams and Moss, 1995). Wong et al. (1988) reported a generation time of 27 minutes in pasteurized milk at 86°F. Johnson et al. (1983), reported a generation time of 26 to 57 minutes at 86°F in cooked rice. Beuchat et al. (1980), reported a generation time of 18-27 minutes in laboratory media. The variability in growth and generation time is dependent on strain, temperature, and nutrient supply of the media. The organism will grow significantly better in dishes containing beef, chicken, or egg in combination with rice, (products containing various nutrients) than in plain rice.

Plain rice often contains 100 vegetative cells per gram as well as spores of *B. cereus*. This population is capable of multiplying to 10⁵ microorganisms in as little as 300 minutes (5 hours) at 86°F.

Nutrients. *Bacillus cereus* produces enzymes than can hydrolyze (split) starch and protein. It thus uses these products of hydrolysis (i.e., sucrose, maltose, lactose, mannose, acids) for growth and reproduction.

pH. The pH range for the organism's growth is 4.3 to 9.3. In meat, *B. cereus* grows at a pH as low as 4.35.

Atmosphere. *Bacillus cereus* is aerobic but can be facultatively anaerobic. Emetic strains can produce sufficient numbers of microorganisms and enterotoxin (in 12 hours at room temperature) in an aerobic environment to cause illness, long before spoilage is evident.

Water activity. The minimum a_w reported for the growth of *B. cereus* is 0.91 to 0.96 in fried rice (Bryan et al., 1981)

Spores

Like all spores, the spores of *B. cereus* are resistant to heat and will survive both cooking and baking temperatures. The time required to inactivate spores is dependent on temperature, strain, media, and food. The thermal destruction time for spores suspended in skim milk at 212°F is 2.7 to 3.1 minutes (Kramer et al. 1989). Time required for destruction of spores in rice varies from 22 to 36 minutes. Fat has a protective effect on the spores of *B. cereus*. If oil is present, spore survival may exceed 30 minutes.

Individual spores may possess an unusually high heat resistance. Hence, in samples of 10⁵ to 10⁶ spores, 1 may survive after 4 hours at 275°F (Franklin, 1970). Spores of *B. cereus* have been isolated from commercially canned foods (Bradshaw et al., 1975). Spore germination is inhibited by carbon dioxide and bicarbonate.

Spores survive in slowly heated rice dishes, casseroles, and meat loaves. If slow cooling follows, spores will outgrow and produce vegetative cells. Once the vegetative cells have been produced from spores of either emetic or diarrheal strains of *B. cereus*, the generation time is approximately 26 to 57 minutes

(Wong et al., 1988, Johnson, 1984) depending on the food supply and temperature.

Vegetative cells sporulate (form spores) when growth conditions are less than optimum (during slow heating, cooling, and changes in other environmental conditions such as pH or acidity). In this manner, the microorganisms are able to survive.

Toxin Production

Bacillus cereus forms enterotoxins, which produce two different types of illness. Certain strains produce enterotoxins that cause diarrheal-type illness and other strains produce enterotoxins that cause the emetic (vomiting)-type illness.

Diarrheal illness due to *B. cereus* is often associated with meat products, soups, potatoes, starchy vegetables, pudding, and sauces. The emetic syndrome is most often associated with rice and pasta products that were held at improper holding temperatures or cooled too slowly.

The enterotoxin associated with diarrheal illness is easily destroyed by heat: (D 132.8°F = 5 minutes. The enterotoxin associated with the emetic illness is quite heat resistant and is stable to 250°F for 90 minutes (Johnson, 1984).

Infective Dose

In order to produce sufficient amount of toxin or sufficient number of cells to cause illness when food is ingested there must be more than 5×10^5 cells per gram of food (Doyle, 1988). The FDA (1993) states that "The presence of large numbers of *B. cereus* in food is indicative of active growth and proliferation of the organism and is consistent with a potential hazard to health."

Symptoms

Symptoms of the diarrheal illness include abdominal pain and profuse watery diarrhea with little vomiting and no fever. Symptoms appear after 10 to 12 hours and usually subside within another 12 hours. The diarrheal illness is often confused with illness caused by *Clostridium perfringens*.

Symptoms of the emetic illness mimic illness symptoms of *Staphylococcus aureus*. They include nausea, vomiting, and possible diarrhea, usually within 1/2 hour to 6 hours after ingestion. There is no fever and recovery occurs within 6 to 24 hours.

Incidence

As a cause of foodborne illness, *B. cereus* was first reported from Norway in 1950. The first well-documented incident in the U.S. occurred in 1969. Meat loaf contaminated with 7×10^6 *B. cereus*/g was the vehicle for transmission of the diarrheal illness involving 15 people.

Outbreak Example. The following example appeared in MMWR 35(25):408-410, 1986.

Bacillus cereus -- Maine. On September 22, 1985, the Maine Bureau of Health was notified of a gastrointestinal illness among patrons of a Japanese restaurant. Because the customers were exhibiting symptoms of illness while still on the restaurant premises, and because uncertainty existed as to the etiology of the problem, the local health department, in concurrence with the restaurant owner, closed the restaurant at 7:30 p.m. that same day.

Eleven (31%) of the approximately 36 patrons reportedly served on the evening of September 22 were contacted in an effort to determine the etiology of the outbreak. Those 11 comprised the last 3 dining parties served on September 22. Despite extensive publicity, no additional cases were reported.

A case was defined as anyone who had vomiting or diarrhea within 6 hours of dining at the restaurant. All 11 individuals were interviewed for symptoms, time of onset of illness, illness duration, and foods ingested. All 11 reported nausea and vomiting; 9 reported diarrhea; 1 reported headache; and 1 reported abdominal cramps. Onset of illness ranged from 30 minutes to 5 hours (mean 1 hour, 23 minutes) after eating at the restaurant. Duration of illness ranged from 5 hours to several days, except for 2 individuals still symptomatic with diarrhea 2 weeks after dining at the restaurant. Ten persons sought medical treatment at local emergency rooms on September 22. Two ultimately required hospitalization for rehydration.

Analysis of the association of food consumption with illness was not instructive, since all persons consumed the same food items: chicken soup; fried shrimp; fried zucchini, onions, and bean sprouts; cucumber, cabbage, and lettuce salad; ginger salad dressing; hibachi chicken and steak; and tea. Five people ordered hibachi scallops, and 1 person ordered hibachi swordfish. However, most individuals sampled each other's entrees.

One vomitus specimen and 2 stool specimens from 3 separate individuals yielded an overgrowth of *B. cereus* organisms. The hibachi steak was also culture-positive for *B. cereus*, although an accurate bacterial count could not be made because an adequate amount of the steak remained for laboratory analysis. No growth of *B. cereus* was reported from the fried rice, mixed fried vegetables, or hibachi chicken.

According to the owner, all meat was delivered 2 to 3 times a week from a local meat supplier and refrigerated until ordered by restaurant patrons. Appropriate-sized portions for a dining group were taken from the kitchen to the dining area and diced or sliced, then sautéed at the table directly in front of restaurant patrons. The meat was seasoned with soy sauce, salt, and white pepper, open containers of which had been used for at least 2 months by the restaurant. The hibachi steak was served immediately after cooking.

The fried rice with the meal was reportedly customarily made from leftover boiled rice. It could not be established whether the boiled rice had been stored refrigerated or at room temperature.

The emetic syndrome has almost always been associated with fried rice served in Oriental restaurants. The common practice of storing boiled rice at room temperature for subsequent preparation of fried rice has generally been implicated in such outbreaks. However, a recent, well-documented outbreak of the emetic syndrome of *B. cereus* in a British prison implicated beef stew. This was thought to be caused by adding to the stew vegetables that were cooked a day earlier.

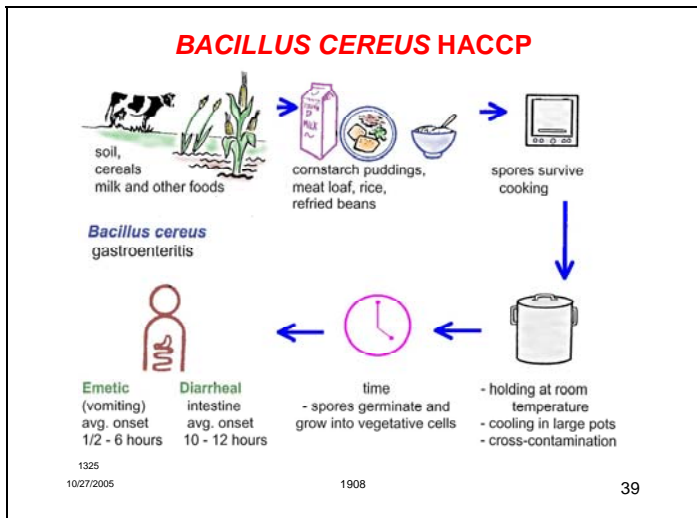
Fresh meat cooked rapidly, then eaten immediately, seems an unlikely vehicle for *B. cereus* food poisoning. The laboratory finding of *B. cereus* in a food item without quantitative cultures and without accompanying epidemiological data is insufficient to establish its role in the outbreak. A negative culture of fried

rice eaten with the meal does not exclude the obvious vehicle; reheating during preparation may eliminate the bacteria in the food without decreasing the activity of the heat-stable toxin. While the question of the specific vehicle remains incompletely resolved, the clinical and laboratory findings substantially support *B. cereus* as the cause of the outbreak.

Most episodes of food poisoning undoubtedly go unreported, and in most of those reported, the specific pathogens are never identified. Alert recognition of the clinical syndrome and appropriate laboratory work permitted identification of the role of *B. cereus* in this outbreak.

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Bacillus cereus - Process Hazard Analysis and Critical Controls

Transmission

Bacillus cereus is widely distributed in nature. It is present in many food ingredients and products. Its spores are able to survive long-term dry storage conditions and are resistant to heat encountered in many cooking methods. Factors leading to incidents of *B. cereus* foodborne illness outbreaks include: inadequate cooling, preparation of food too far in advance, infected food handlers (people carry the microorganisms), and slow and inadequate heating of products.

For example, tortillas and burritos are often contaminated with *B. cereus*. The source of contamination can be the flour and other grains used to prepare tortillas and the beans, meat, spices, or cheese used to fill the tortillas. If these products are allowed to remain at temperatures that promote the growth and toxin production of *B. cereus*, they will become hazardous.

Another example of a potentially hazardous procedure is the soaking of dried beans, peas, and other legumes in hot water overnight before cooking them. This practice allows the spore germination of emetic strains of *B. cereus*, growth of vegetative cells, and production of the very heat-resistant enterotoxin. Although cooking of the beans will destroy the vegetative cells, the toxin will remain in these products to cause foodborne illness. A more acceptable procedure is to pour hot water over the legumes, bring them to a boil, turn off the heat and let them soak for 1 hour. Then, the heat can be turned on again to cook them until they are tender or reach the desired state of doneness.

In addition to starches and cereals, *B. cereus* has been isolated in milk (both raw and pasteurized). Under proper conditions, the organism produces a condition known as "broken cream" or "bitty" cream in both raw and pasteurized milk. The cream and the milk protein casein are degraded and cannot be re-emulsified. This gives the appearance of "curdling" or "souring" when such dairy products are used in coffee and tea.

Bacillus cereus has been isolated from fecal material of healthy humans. It must be accepted that the organism is present in food and is not dangerous when healthy persons ingest small numbers. Illnesses occur when large numbers of cells are

ingested in foods and/or produce toxins in food products that are not destroyed in reheating.

Control

Food production and foodservice establishments must:

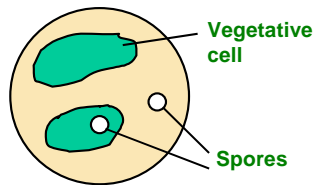
1. Use cooking methods that destroy vegetative cells and most spores. Since the vegetative cells of *B. cereus* are more easily destroyed than *Salmonella* spp., *Salmonella* pasteurization temperatures will inactivate *B. cereus* vegetative cells. These methods include: steaming under pressure, frying, roasting, and grilling. These methods do not render these products free of spores, but they are more effective in reducing the spore population than are other shorter, lower temperature cooking methods and microwave cooking.
2. Prepare products, particularly rice, pasta, and other cereal products as close to service time as possible. If foods are prepared in advance, they should be cooled according to FDA recommendations or USDA Guidelines or held above 130°F for safety (135°F, FDA recommendations). Cooked food products should not be stored at room temperature.
3. Store or hold cooked, pasteurized food products according to the table on page 4-2.
4. Reheat leftovers and/or partially prepared items to 165°F or above. **Reheating food to 165°F or above eliminates vegetative cells, however, it does not inactivate the spores of *Bacillus cereus* nor destroy emetic toxin if it has formed.**
5. Cool food in layers that are less than 2 inches deep in front of a high velocity fan, so that rapid cooling occurs. Foods should not be cooled in large, deep pans or kettles.
6. Train foodservice personnel to use good personal hygiene and proper methods of hand washing when handling food products. People can be carriers / shedders of this microorganism.
7. Check temperatures in hot holding devices and in cooling units to assure that this equipment functions properly and repair or replace it as needed.
8. Use methods that adequately clean and sanitize surfaces, equipment, and utensils.

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CHARACTERISTICS OF *CLOSTRIDIUM BOTULINUM*

- Grows without air (oxygen) in canned foods and vacuum packages.
- Common in soil.
- Proteolytic types A and B growth: 50 to 118°F.
- Non-proteolytic types B and E growth: 38 to 113°F.
- Spores survive boiling temperatures to outgrow as vegetative cells that grow and produce toxin in little or no air (oxygen).
- Neurotoxins are deadly.
- Toxin(s) destroyed: 185°F for 5 minutes.



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Clostridium botulinum - Characteristics Bacterial Characteristics

Clostridium botulinum microorganisms are anaerobic, spore-forming, gram-positive rods that are motile by means of flagella. There are seven types of *C. botulinum*, A, B, C, D, E, F, and G. Human botulism is principally caused by types A, B, and E. Types F and G have caused extremely few, rare cases of human botulism. Types C and D cause botulism in birds and animals.

Botulism is currently classified into four categories: (Pierson and Reddy, 1988; FDA, 1993)

1. Classical foodborne botulism intoxication caused by the ingestion of small amount of preformed botulinum toxin in contaminated food.
2. Wound botulism (a rare occurrence), which results from the growth of *C. botulinum* and production of toxin in infected wounds.
3. Infant botulism, is thought to be caused by the ingestion of *C. botulinum* spores that colonize and produce toxin in the intestinal tract of infants. At this time, honey is the only implicated food source of *C. botulinum* spores. (Parents of newborn infants are now warned not to give honey to babies.) Only infants under 1 year of age are affected.
4. Undetermined cause of botulism that involves individuals older than 12 months of age. It has been suggested that some cases of botulism in adults assigned to this category might be the result of intestinal colonization and resultant toxin production within the gut. In these cases, patients had surgical alteration of the gastrointestinal tract and/or antibiotic therapy that may have altered the normal gut microflora and allowed *C. botulinum* to colonize the intestinal tract.

Most cases of human botulism are due to the growth of types A, B, and E *C. botulinum* in food and subsequent production of neurotoxins. Ingestion of food containing the neurotoxins causes severe illness and possibly death, if antitoxins are not administered promptly.

Type A *C. botulinum* and some strains of type B are proteolytic (capable of splitting proteins into their constituent amino acids). The growth of proteolytic strains of *C. botulinum* produces off flavors and odors in food and food products. Type A and

proteolytic type B may be present on meat and vegetables, particularly those growing in or near the ground.

Type E *C. botulinum* is most often associated with fish and seafood products. Type E strains are found in the water and sludge near bodies of water (i.e., oceans, lakes, and rivers.) Type E *C. botulinum* and non-proteolytic strains of type B *C. botulinum* are capable of growing at refrigeration temperatures. Because both of these strains are non-proteolytic, they can grow and produce toxin in food without changing its flavor and odor.

The vegetative cells of these microorganisms are destroyed when products are heated during most cooking procedures. However, the spores can survive most cooking procedures and will germinate to form vegetative cells if these products are kept in anaerobic conditions at temperatures that support growth. When vegetative cells grow in these anaerobic conditions, a lethal neurotoxin is produced.

Source

Spores and vegetative cells of *Clostridium botulinum* are present in soil, water, and sludge near bodies of water. The number of foods in which types of *C. botulinum* have found is limitless.

Growth Conditions

Temperature. Type A and proteolytic type B strains of *C. botulinum* grow at temperatures between 50 to 118°F (Hauschild, 1989). Ohye and Scott (1953) found the optimal temperature range for growth to be 98.6 to 104°F. At 98.6°F, the generation time is 0.7 hours (42 minutes). The following table indicates the generation times for these strains at various temperatures.

Gibson et al. (1987) reported a generation time for *C. botulinum* type A in pasteurized pork slurry of 1.2 hours at 68°F.

The temperature range for growth of type E and non-proteolytic type B strains is 38 to 113°F. This means that botulism can occur from ingestion of refrigerated foods, if they are stored for long periods of time (14 to 21 days) in anaerobic conditions.

INFLUENCE OF TEMPERATURE ON THE GROWTH OF *CLOSTRIDIUM BOTULINUM* TYPES A AND B*

Temperature °F	Approx. lag time (hour)	Approx. generation time (hour)
54	**	89
59	160	28
64	63	8
68	32	4
77	20	2
86	8	1
98.6	5	0.7
108.5	8	1
113	**	2

* Adapted from data of Ohye and Scott (1953) as reported by Sperber (1982). (The cultures used in this study were grown in laboratory media of neopeptone, yeast extract, glucose, and water.)

** Not reported.

Freezing decreases the number of vegetative cells in foods but has no effect on spores or preformed toxins.

Nutrients. These organisms require a supply of amino acids (protein), purines, polyalcohols, and sugars (glucose or maltose

is known to be needed for toxin production), (Frazer, 1978 and Ketchum, 1984).

pH. The pH range for growth of type A and proteolytic types of *Clostridium botulinum* is 4.6 to 9.0. It has been reported that these strains will grow in at pH as low as 4.0. In these instances, either yeasts or molds grew in areas of the food, increasing the pH in these areas to a level that allowed spore germination and vegetative cell growth. Tanaka (1982) reported that toxin was produced in a pork slurry at a pH of 4.30 to 4.36. He postulated that *C. botulinum* was able to grow and produced toxin at a higher pH within precipitated protein matrices.

Types E and nonproteolytic strains of type B. *C. botulinum* grow in a pH range of 5.0 to 9.0.

Atmosphere. *Clostridium botulinum* is an anaerobe, but may grow under certain conditions of reduced oxidation-reduction potential. It is capable of growth in the absence of air in canned products, vacuum packaged products, and in the interior portions of food products from which oxygen has been eliminated by cooking. Exclusion of oxygen inhibits aerobic microorganisms such as the lactic acid bacteria, which, when air is available, tend to suppress growth of *C. botulinum*.

Water Activity. The water activities, effective in inhibiting the growth of vegetative cells, are about 0.94 for type A and nonproteolytic strains of type B, *C. botulinum*, and 0.97 for type E and nonproteolytic strains of type B, *C. botulinum*. A salt (sodium chloride) concentration of 10% in brine is effective in lowering the water activity to 0.94, a brine concentration of 5% salt is effective in reducing the water activity to 0.97 (Hauschild, 1989).

Other Factors. Competitive microorganisms have a protective effect in foods by inhibiting the growth of *C. botulinum* and by causing spoilage in food products before a significant population of *C. botulinum* can grow and produce toxin.

Nitrites added to cured meat, poultry, and fish products inhibit the growth of *C. botulinum*. The effectiveness of nitrites is enhanced by the addition of acidity, salt, and isoascorbate.

Liquid smoke products, which are commonly applied to the surface of many cured meat and fish products, also inhibit the growth of this microorganism. Smoking of products in a smoke house has little prohibitive effect.

Nisin, an antibiotic, is used to inhibit the growth of *C. botulinum* in cheese spreads.

Heat Resistance of Vegetative Cells and Spores

The vegetative cells of *C. botulinum* are readily inactivated by most cooking methods. However, both spores and/or toxins may survive if heating is not adequate. The ingestion of vegetative cells and spores does not cause illness (except in infants and certain individuals whose intestinal microflora does not inhibit the growth and toxin production of *C. botulinum*).

The primary consideration for safety in food preservation is the destruction of *C. botulinum* spores in the processing of food products. The heat resistance of spores varies among types. Spores of proteolytic types or strains (type A, proteolytic types B and F) require 0.3 to 0.23 minutes for 90% reduction of spores at 250°F [D₂₅₀]. Spores of nonproteolytic types E, B, and F are less

heat resistant and are destroyed at 180°F [D₁₈₀] in 0.8 to 6.6 minutes in various foods (Simunovic et al., 1985).

The following table summarizes some growth conditions for *C. botulinum*.

MINIMAL REQUIREMENT FOR GROWTH AND HEAT RESISTANCE OF CLOSTRIDIUM BOTULINUM TYPES A, B, E, AND F*

Properties	Group	
	I (Proteolytic)	II (Non-proteolytic)
Toxin types	A, B, F	B, E, F
Inhibitory pH	4.6	5.0
Inhibitory salt (NaCl) concentration	10%	5%
Minimal water activity	0.94	0.97
Temperature range for growth	50-118°F	38-113°F

* Adapted from Hauschild, 1989.

Commercial canning procedures are designed to destroy *C. botulinum* spores and make the survival of any spores extremely rare. The canning industry has adopted the 12D concept for heat processing low-acid canned foods (meats, vegetables, and any other products with a pH above 4.4 to 4.6). The 12D process is intended to reduce a bacterial spore population from 1,000 spores in each billion cans of food to 1 spore in 1 billion cans. This heat processing method has been quite effective in providing a safe supply of canned food. Incidents of botulism due to the consumption of commercially canned foods has been due to inadequate heat processing (inaccurate retort temperatures and timing) and/or recontamination after processing through leaks of cooling water through side seams in cans of food.

Toxin Destruction

The neurotoxins produced by all types of *C. botulinum* are less heat resistant than the enterotoxins produced by *Staphylococcus aureus*. Woodburn et al. (1979) found that any botulinum toxin was inactivated if it was heated to 174°F for 20 minutes [D₁₇₄ = 20 minutes] or 185°F for 5 minutes [D₁₈₅ = 5 minutes].

Many food products involved in documented botulism incidents were not heated or were heated insufficiently after toxins have been produced to inactivate the toxins.

Symptoms

Symptoms of this foodborne intoxication develop within 12 to 72 hours after consumption of the toxin-containing food. Symptoms include: nausea, vomiting, fatigue, dizziness, headache, skin dryness, dryness of the mouth and throat, constipation, paralysis of muscles, double vision and difficulty in breathing. Duration and severity of the illness is dependent upon the amount of toxin ingested and the overall health of individuals. Treatment involved administration of anti-toxin and respiratory therapy. Death results in 10% of diagnosed cases.

Toxic Dose

The botulinum toxins are the most toxic substances known. A very small amount (a few nanograms) in food can cause illness. Botulinum toxin is produced when cells grow in suitable environmental conditions. Type A toxin has been reported to be

more lethal than types B or E toxins (Jay, 1996). A bacterial cell population of 10^4 to 10^5 cells/gram of food is required to produce sufficient toxin.

Incidence

The word botulism is derived from the word botulus (Latin for sausage). A German scientist, E. P. M. van Ermengen in 1896, isolated the microorganism in inadequately cured ham. The ham had caused illness in 34 people, which resulted in 3 deaths. He named the microorganism *Bacillus botulinus*, because this illness had the same disease symptoms as blood sausage poisoning. The organism was later renamed *Clostridium botulinum*.

Most botulism outbreaks in the United States have been associated with vegetables. However, fish and seafood products, meat products (beef, pork, and poultry), condiments (chili sauce, tomato relish, and salad dressing), and dairy products have also been causes of incidents. Between 1899 and 1976, home-processed foods were responsible for the majority of the incidents (72%), while commercially processed foods were involved in about 8.6% outbreaks. Unknown vehicles were responsible for the other 20% of outbreaks.

In 1960 and 1963, 4 outbreaks of type E botulism occurred in the United States involving 23 cases resulting in 9 deaths. These outbreaks reactivated the interest in botulism. Three outbreaks of type E botulism were traced to fish or fish products; the other 1 was traced to canned mushroom sauce. (Canned food has seldom been involved in type E outbreaks, but canned sprats, mushroom sauce, and tuna fish have been involved.) In the canned tuna fish outbreak in Detroit, Michigan, 1963, the product was apparently contaminated after retorting because of faulty can seams. In 1963, 2 other outbreaks of type E botulism occurred from commercially prepared smoked whitefish chubs originating from the Great Lakes.

After 1970, a variety of commercially canned foods (vichyssoise, peppers, marinated mushrooms, and beef stew) were found to be contaminated with types A or B, *C. botulinum*. Consumption of these products caused 13 clinical cases of botulism, including 2 deaths in the United States and Canada. During 1973 and 1974, a total of 30 cans of mushrooms were found to contain type A *C. botulinum* toxin. Each year from 1980 to 1988 the number of cases of foodborne botulism reported to the CDC in Atlanta, Georgia, ranged between 17 to 50 cases a year. During that same period of time, there were 50 to 100 cases of infant botulism per year.

Food Analysis

Botulism is foodborne and usually results from ingestion of the preformed toxin. Therefore the source of an outbreak is based on detection and identification in the food involved. The most widely accepted method is the injection of extracts of the food into passively immunized mice (mouse neutralization test). The test takes 48 hours. This analysis is followed by culturing all suspect food in an enrichment medium for the detection and isolation of the microorganism. This latter test takes 1 week.

Outbreak Examples. The following outbreak example appeared in MMWR 32:39-45, 1983.

Botulism and Commercial Pot Pie -- California. On August 3, 1982, a 56-year-old woman residing in Los Angeles County,

California, developed diplopia, weakness, difficulty breathing, and chest pain. She had respiratory arrest on admission to the hospital but was intubated, resuscitated, and placed in intensive care. Examination showed complete bilateral ptosis, ophthalmoplegia, facial muscle weakness, and areflexia. Cerebrospinal fluid was normal except for increased glucose; tensilon test was negative. She had a history of seizure disorder, diabetes mellitus, and organic brain syndrome. An infectious disease consultant thought her subsequent fever was due to pneumonia secondary to aspiration, and he suspected botulism as the underlying cause of her illness.

The patient lives with her husband and grown son who both prepare meals for her and attempt a strict diet in consideration of her diabetes. When asked about the patient's food history before onset of illness, the husband and son named no likely suspects for botulism. No home-preserved foods had been served, and, with one exception, she had not eaten other foods that were not freshly prepared for her or were not also consumed by her husband and son. The exception was commercial beef pot pie, which was accidentally mishandled, then consumed by the patient one day before illness began.

The son had prepared the pot pie for an earlier evening meal. The frozen pie was baked in an oven for 40-45 minutes. As he was about to serve it to his mother, his father came home with some freshly cooked hamburgers just purchased at a take-out restaurant. The pot pie was put aside on an unrefrigerated shelf. Two and one-half days later, the son came home and found his mother had just consumed this pot pie without reheating it.

An uneaten portion of the pot pie, still in its metal plate, was retrieved by the family members. Type A botulism toxin was found in this pie by a mouse-inoculation test performed at a U.S. Department of Agriculture laboratory in Beltsville, Maryland, and type A toxin was also demonstrated in the patient's serum by the state's Microbial Disease Laboratory.

This is the third case of botulism associated with commercial pot pies reported from California; 1 other episode involving 2 clinically diagnosed patients was reported from Minnesota in 1960. Mishandling of the pot pies occurred in three of these episodes, and mishandling was also suspected in the fourth. The known mishandlings consisted of leaving the baked pot pie in the oven with the pilot light on, thereby maintaining "incubatory" temperatures overnight. The pies were then eaten with no reheating to destroy toxin. Or, as in the present case, the baked pie sat out at room temperature for over 2 days during hot weather -- conditions that also could simulate an incubator.

In these situations, it is suspected that the original baking killed competing organisms in the pies and eliminated much of the oxygen. The heat-resistant, anaerobic *C. botulinum*, which was evidently present and can be found in many fresh, frozen, and other food products, was then presumably able to germinate and produce toxin under the crust during storage at warm, incubator-like temperatures. Products such as pot pies should be kept frozen before heating and ideally should be served hot after the first cooking. If any such products are to be saved, they should be quickly refrigerated, then reheated to hot temperatures. This would minimize any risk of botulinal poisoning.

Other Examples. Foodservice establishments have also been involved in botulism incidents. In November 1978, 7 cases of

type A botulism occurred in persons who had eaten in a restaurant in Colorado (Seals et al., 1981). The outbreak was recognized when 2 persons who had eaten at the restaurant were hospitalized with botulism; 5 additional cases were reported. Potato salad made at the restaurant and served during an 11-day period was incriminated as the vehicle of transmission. The potato salad had been prepared from potatoes baked for service in aluminum foil. The potatoes were "left-over" and were allowed to remain in the foil-wrapping at room temperature before being used to prepare potato salad. Laboratory studies confirmed that *C. botulinum* spores on the surface of the potatoes could survive baking and that botulinum toxin could be produced in potatoes contaminated with *C. botulinum* spores in sealed aluminum foil wrappers if these products were held at ambient temperatures for 1 day or less (Sugiyama et al., 1981).

In October of 1983, 28 people were hospitalized in Illinois with neurological signs and symptoms of botulism. Twelve patients required ventilatory support, and 20 patients were treated with antitoxin. One patient died 6 months after the onset of the illness. Type A toxin and/or type A *C. botulinum* was identified from specimens of 18 patients. Case control studies implicated sautéed onions made from fresh raw onions, served on a patty melt sandwich in a local restaurant as the vehicle of transmission. Type A toxin was detected in washings from a wrapper in which a patty-melt sandwich was taken home by one of the ill persons. Type A *C. botulinum* was also cultured from 5 of 75 raw onions taken from the restaurant. Onions used to prepare the patty-melt sandwiches had been partially cooked in butter a day previous to making the sandwiches. They were placed in a pan, covered with a layer of melted butter, and were stored on a warm counter on the back of the grill for 12 to 24 hours before the sandwiches were prepared. These storage conditions were optimal for growth of *C. botulinum* and subsequent toxin production (MacDonald et al., 1985).

Between July 26 and September 5, 1985, 37 cases of type B botulism were caused by food served at a restaurant in Vancouver, British Columbia. Seven persons required mechanical ventilation. *C. botulinum* type B toxin was found in the serum of 3 patients, and type B spores were found in cultured feces of 1 patient a month after the outbreak. Commercially bottled chopped garlic in soybean oil was implicated by the Centers for Disease Control as the food vehicle in this outbreak. Although the product involved was labeled "Keep Refrigerated" in very small print, the garlic jar at the restaurant was kept at room temperature (Solomon and Kauter, 1988).

An international outbreak of type E botulism associated with ungutted, salted whitefish occurred in the fall of 1987 (Centers for Disease Control, 1987). In November of 1987, a Russian immigrant and his 9-year-old son were admitted to a suburban New York hospital with symptoms indicative of botulism. The father's stool specimen contained type E botulinum toxin. The father had purchased a whole, ungutted, salted, air-dried whitefish known as kapchunka from a delicatessen in Queens, New York City. He and his son ate the fish a week later and both became ill within the next day. At the same time, the Centers for Disease Control in Atlanta, Georgia, received a report from the Ministry of Health, Jerusalem, Israel, of 5 additional cases suspected to be botulism. One case was fatal. The patients had eaten whitefish purchased at a grocery in Brooklyn, New York City in the middle of October 1987 and

then taken to Israel. The fish as well as a serum sample from 1 surviving patient yielded type E botulinum toxin.

Kapchunka is an ethnic food consumed in this country primarily by Russian immigrants. In 1981, a California man became ill, and, in 1985 2 Russian immigrants died in New York City after consuming this fish product.

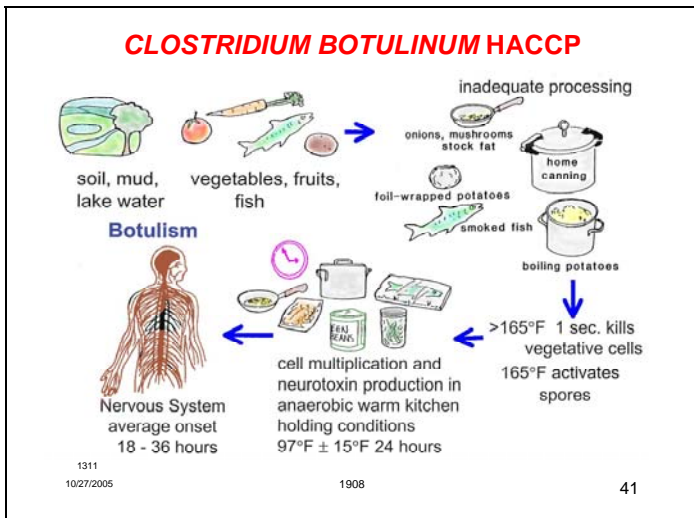
In the fall of 1988, 3 carnival workers in Louisiana became ill with botulism after consuming cole slaw, purchased at a delicatessen, that was allowed to remain unrefrigerated for 3 days.

These incidents are examples of improper processing and/or storage of food products that allowed spores of *C. botulinum* to germinate into vegetative cells that multiplied and produced toxin in foods. Botulism resulted when these foods were consumed and fatalities occurred.

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Clostridium botulinum - Process Hazard Analysis and Critical Controls

Clostridium botulinum Transmission

Spores and vegetative cells of *Clostridium botulinum* are present in the soil and sediment from rivers, lakes and oceans and are therefore present on many of the food products harvested from these sources. These foods include: fresh and canned vegetables; foil-wrapped, unrefrigerated baked potatoes; unrefrigerated, wrapped, or vacuum-packed fish; keep-refrigerated, cooked or partially cooked convenience food items; and imported canned products (e.g., antipasto, smoked salmon and other seafoods). Liver pate and ham have also been implicated in botulism outbreaks because meat is slightly contaminated with types A and B *C. botulinum* spores.

In general, the presence of *C. botulinum* and/or its toxins in canned foods indicates faulty processing. Inadequately processed foods such as meats and vegetables (green beans, corn, beets, asparagus, and spinach), particularly home-canned items, have often been associated with botulism.

Frozen foods have not been associated with outbreaks of botulism. The vegetative cells do not grow at freezing temperatures. However, spores of *C. botulinum* may survive in foods during long freezer storage periods. After the food has been thawed, these spores can form vegetative cells. The cells will grow and produce toxin in foods if environmental conditions of temperature, pH, and atmosphere provide favorable growth conditions.

Spores of *C. botulinum* cannot germinate in foods with a pH lower than 4.6. Botulinal toxin can be produced in food in 1 to 10 days. Growth and subsequent toxin production is based on temperature, atmosphere, and other environmental conditions.

Commercial Sterilization

Commercial sterilization refers to the heat treatment given various processed foods. This "sterilization" process does not mean that these foods are free of bacterial spores. It does mean that a sufficient heat treatment has been given to these products to reduce a hypothetical population of 1 billion spores in each of 1,000 cans to only 1 spore per 1,000 cans. Low-acid foods (vegetables, meat, fish, and poultry items) require processing at

elevated temperatures for sufficient periods of time in order to ensure the destruction of botulinal spores.

Some products (e.g., beets, cucumbers) are acidified with acetic acid (vinegar) prior to processing. This acidification permits the use of milder preservation heat treatments. It is essential that the pH of such acidified foods be permitted to equilibrate thoroughly before the heat process is applied. This requires sufficient acid, stirring and time for the pH to decrease to 4.6 or below at the center of solid materials. Most fruits are naturally acid, and are processed at temperatures that do not exceed 212°F. Since spores of *C. botulinum* do not germinate in acid foods (pH below 4.4-4.6), processing times and temperatures are based on the destruction of vegetative cells of microorganisms that cause spoilage in these products.

Detection

Food containing botulinal toxin in any amount is unacceptable. Excellent methods are available for detection of *C. botulinum* and its toxins. They are invaluable for the examination of foods implicated in botulism outbreaks and for other investigational purposes. However, the expertise required in application of the methods and in the interpretation of results precludes their use in most laboratories that routinely analyze food. The probability that the examination of a reasonably sized sample of low-acid canned food contaminated with *C. botulinum* would result in detection of the organism is too low to assure the level of safety necessary. The safety of low-acid canned foods depends primarily on instrumentation and adequate process mechanisms to provide assurance that processing is adequately accomplished and that container integrity is maintained.

Control

Control of the botulism hazard in perishable foods must be based on adherence to food handling practices that prevent the growth of *C. botulinum*. In order to prevent outbreaks of botulism in foodservice:

1. Canned foods should be rejected if they exhibit defects such as swelling, rust, and/or leakage. These types of food must not be used to prepare any food items for retail food use.
2. Canned goods should be stored under conditions recommended for these items.
3. Any prepared food products that have received a light heat treatment and are labeled "keep refrigerated" and perishable fish items (e.g., smoked fish products) must be stored at 37°F or below.
4. Fresh vegetables (e.g., mushrooms) must be packaged in containers or bags that allow air (oxygen) to enter the packages when they are stored at temperatures of 50°F or above.

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