These strains serve a useful function in the body by being harmless and are a part of the normal intestinal microflora of man and warm-blooded animals. Most strains of *Escherichia coli* are 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.

Vegetative cells killed by cooking / pasteurization.

Escherichia coli – Characteristics

**Bacterial Characteristics**

*Escherichia coli* are gram negative, non-spore-forming rods. Some may or may not be mobile. (Some rods are flagellated and some are not.) The organisms are facultative anaerobes that ferment 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 (37°C), with a range of 36.5 to 114°F (2.5 to 45°C). Table 4-14 indicates the generation times for *E. coli* O157:H7.

<table>
<thead>
<tr>
<th>Temperature °F (°C)</th>
<th>Generation Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>36 (2.2)</td>
<td>no growth</td>
</tr>
<tr>
<td>77 (25)</td>
<td>87.6</td>
</tr>
<tr>
<td>86 (30)</td>
<td>34.8</td>
</tr>
<tr>
<td>99 (37.2)</td>
<td>30.0</td>
</tr>
<tr>
<td>104 (40)</td>
<td>38.0</td>
</tr>
<tr>
<td>111 (43.9)</td>
<td>65.0</td>
</tr>
<tr>
<td>113 (45)</td>
<td>72.6</td>
</tr>
<tr>
<td>114 (45.6)</td>
<td>no growth</td>
</tr>
</tbody>
</table>

*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).

The minimum water activity (a_w) for growth of *E. coli* O157:H7 is 0.95

*Escherichia coli* O157:H7 can survive in ground beef at -4°F (-20°C) 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 (10³ to 10⁵ 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 10³ to 10⁵ *E. coli* / gram. These strains are 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 that 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 10³ to 10⁵ 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 that 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 (HUS) in children. This is the leading cause of...
kidney failure in children that often requires kidney 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.

The first documented outbreaks of this pathogenic bacteria in food in the United States occurred in 1982 in Oregon and in Michigan. Both outbreaks were traced to fast food restaurants of the same chain. Infected individuals had eaten contaminated hamburgers. Investigations revealed that frozen ground beef patties had not been heated sufficiently to inactivate E. coli O157:H7 found to be present in the ground meat.

In the fall of 1988, an incident occurred at a junior high school in Minnesota that lead 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 a 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 recommends that all ground meat products be cooked until all parts of the food reach a temperature of 155°F (68.3°C) for 15 seconds. The color of meat products be cooked until all parts of the food reach a temperature of 155°F (68.3°C) for 15 seconds. The color of meat products be cooked until all parts of the food reach a temperature of 155°F (68.3°C) for 15 seconds. The color of meat products be cooked until all parts of the food reach a temperature of 155°F (68.3°C) for 15 seconds.

Other outbreaks of E. coli O157:H7 have been found to be associated with unpasteurized cider and 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. These 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 about 80 fatalities (Mead et al., 1999).

**References:**


**Escherichia coli – Process Hazard Analysis and Critical Controls**

**Transmission**
Raw foods, particularly those of animal origin such as meat and unpasteurized dairy products, can be contaminated with *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. Fresh fruits and vegetables, become contaminated when polluted water is used for irrigation.

Carcasses are often contaminated with fecal material of infected animals, or from other contaminated carcasses, or equipment. 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**
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 Table 4-15, the D values at 125°F (51.7°C), 135°F (57.2°C) and 145°F (62.8°C) are based on this research article. The FDA used this data to extrapolate mathematically the destruction values for *E. coli* O157:H7 at 140°F (60°C), 145°F (62.8°C), 150°F (65.6°C) and 155°F (68.3°C). The z-value for these sets of data is approximately 8.3°F.

Using the data from these sources, Table 4-15 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).

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.
3. Heating ground meat products, according to thermal inactivation standards given by Table 4-15, or to 155°F (68.3°C) or above, for 15 seconds as recommended by the FDA Food Code. (This is a 5 D *Salmonella* kill.)
4. Separating raw food preparation areas from cooked food preparation areas, and cleaning and sanitizing equipment and surfaces to prevent cross-contamination.
5. Using pasteurized juices and dairy products and irradiated meat and poultry products.
6. Using safe (potable) water for drinking and for washing fruits and vegetables. (This is usually chlorinated tap water from a municipal water supply.)

**References:**


**Table 4-15**

<table>
<thead>
<tr>
<th>Temperature °F (°C)</th>
<th>1 D-value (seconds)</th>
<th>5 D-values (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125 (51.7)</td>
<td>6,930</td>
<td>577.50</td>
</tr>
<tr>
<td>135 (57.2)</td>
<td>318</td>
<td>26.50</td>
</tr>
<tr>
<td>140 (60)</td>
<td>100.1</td>
<td>8.34</td>
</tr>
<tr>
<td>145 (62.8)</td>
<td>25.2</td>
<td>2.11</td>
</tr>
<tr>
<td>150 (65.6)</td>
<td>6.36</td>
<td>0.53</td>
</tr>
<tr>
<td>155 (68.3)</td>
<td>1.56</td>
<td>0.13</td>
</tr>
</tbody>
</table>
Streptococcus – Characteristics

Bacterial Characteristics

The genus *Streptococcus* are gram-positive, spherical-shaped bacteria that grow in chains or pairs. *Streptococcus* can tolerate oxygen, but they are microaerophilic and grow better anaerobically than in air. The genus is defined by a combination of antigenic, hemolytic, and physiological characteristics in groups A, B, C, D, F, and G. Groups A and D can be transmitted in food. Groups A and D can be transmitted in food. Difficult to inactivate with heat; can be an indicator of inadequate pasteurization.

*Streptococcus pneumoniae* is the cause of septic sore throat, scarlet fever and other pyogenic (pus-forming) and septicemic (systemic) infections.

Group D, comprised of *S. faecalis*, *S. faecium*, *S. durans*, *S. avium*, and *S. bovis*, produces symptoms similar to staphylococcal intoxication.

The streptococci are inhabitants of the intestinal tract of humans and animals. When discharged with the feces they may contaminate whatever has contact with the feces, sewage, and manure, such as meat animals, hide, the abdominal cavity of the freshly slaughtered animal, soil, water, air, and the hands of the food handler. Meat may become heavily contaminated with streptococci when sanitary standards in packing plants are low.

Of 5,719 industrially processed food samples analyzed for fecal *Streptococci*, these organisms were recovered from 10.6% of the samples. Frozen foods had a higher incidence than other foods.

Growth

Factors affecting growth vary somewhat with species and strains.

Nutrients. A variety of foods are able to serve as substrates. These organisms in general need complex substrates and grow poorly on many culture media. Their salt tolerance is high (approximately 6.5% or more). *Streptococcus faecium* grows well on salt-cured hams.

pH. The organisms may grow over an extremely wide pH range of 4.0 to 11.0. Especially unusual is their capability to grow in alkaline media of pH 9 and higher.

Temperature. The organisms are capable of growing over a very wide temperature range: 42 to 126°F (5.5 to 52°C) (Mitscherlich and Marth, 1984).

The time-temperature effects on fecal streptococci in foods at refrigeration temperatures as well as at warm holding temperatures were studied in custard, chicken a la king, and ham salad (Angelotti et al., 1963). In custard, growth was slight at 42°F (5.6°C), but more profuse growth was observed at 48°F (8.9°C) through 115°F (46.1°C). In chicken a la king, no growth was noted at 42°F (5.6°C) or below, but profuse growth occurred at the temperature range between 50°F (10°C) and 115°F (46.1°C). In ham salad, no growth occurred at 48°F (8.9°C) and below; at 50°F (10°C) growth was slight; very prolific growth was observed at 60°F (15.6°C) through 115°F (46.1°C).

Some organisms are thermoduric and are able to survive pasteurization of milk. *S. durans* suspended in milk exposed to high temperatures for 16 minutes survived: at 161°F (71.7°C) 55.2% survival; at 170°F (76.7°C) 46.4% survival; and at 180°F (82.2°C) 32.5% survival.

*Streptococcus faecalis* and *S. faecium* have been shown to survive the pasteurization treatment given certain types of canned hams. In general, processors aim at a internal food end temperature of 158°F (70°C) to prevent bacterial spoilage. This temperature may not be sufficient to destroy a high number of these microorganisms. Hence, there is a refrigeration requirement for canned hams.

*Streptococcus faecalis* can remain viable for at least 30 days at 98.6°F (37°C) in foods of varying acidity. At below freezing temperatures the cells remain viable for long periods of time. This property has been claimed to be of some value as a microbiological index of food quality for foods such as water, milk, and frozen foods stored at low temperatures. However, the role of these organisms as indicators of food quality is questionable.

Symptoms of Illness

Group A streptococci are the cause of sore red throat, pain on swallowing, tonsillitis, high fever, headache, nausea, vomiting, malaise, rhinorrhea (nasal discharge), occasionally a rash occurs [FDA, 1993]. The infectious dose is low (less than 1,000 organisms). Culturing of nasal and throat swabs, pus, sputum, blood, suspect food, and environmental samples can diagnose the presence of this pathogen. This type of illness tends to be common in young children. Complications are rare and the fatality rate is low.

Group D streptococci are responsible for diarrhea, abdominal cramps, nausea, vomiting, fever, chills, and dizziness within 2 to 36 hours following ingestion of contaminated food. The illness is acute and self-limiting. The infectious dose is high (greater than 107 organisms) [FDA, 1993]. The presence of this pathogen can be determined by culturing stool samples, blood, and suspect food.

Incidence

There is not much documentation on the incidence of streptococcal foodborne illness in the United States is about 51,000 cases,
resulting in around 350 hospitalizations with no fatalities. (Mead et al., 1999).

**Transmission**

*Streptococcus faecalis* is found in the human intestinal tract. Tests for the presence of this microorganism in food and water have been used to indicate fecal contamination. *Streptococcus faecium* and *S. durans* are found in the intestinal tract of swine. These organisms are found on plants, insects, and in soils. They may be carried to plants by insects, air, or by direct fecal contamination.

Foods and food products that have been suggested as vehicles for transmission of *Streptococcus* microorganisms and causing illness have included: milk, ice cream, steamed lobster, ground ham, pasteurized canned ham, Vienna sausage, beef croquettes, pork sausage, barbecued beef, bologna, turkey dressing, turkey a la king, dried eggs, potato salad, egg salad, shrimp salad, custard, rice pudding, cheese, chocolate pudding, and whipped cream.

In most cases of Group A streptococci transmission, foods were allowed to stand at room temperature for several hours between preparation and consumption. Entrance into the food is usually the result of poor hygiene, ill food handlers, or the use of unpasteurized milk. The presence of Group D streptococci is due to under-processing and/or poor and unsanitary food production or preparation.

Salad bars have been suggested as possible sources of infection by both Group A and D streptococci as the result of poor hygiene and abusive sanitation practices by salad bar patrons.

**Control**

In order to control the presence or amount of Group A and D streptococci in food:

1. Mandate correct hand washing procedures for all food handlers.
2. Buy food products from suppliers who certify the microbiological quality of their products, or heat products to temperatures above 165°F (73.9°C). Table 4-16 indicates the time needed to inactivate *S. faecalis* in 2 food products.

3. Use water from a safe source.
4. Use pasteurized milk and dairy products.

5. Prepare raw foods with separate equipment in a separate area from cooked or partially prepared foods and/or thoroughly clean and sanitize equipment and preparation areas between preparations of raw and cooked foods.

**References:**

Angelotti, R., Lewis, K. H. and Foter, M.J. 1963. Fecal streptococci in foods time-temperature effects on I. Behavior in refrigerated foods and at warm-holding temperatures. J. Milk Food Technol. 26(9): 296-301


**Table 4-16**

**Thermal Inactivation Times for *Streptococcus faecalis* in Fish Sticks and Tuna Pie**

<table>
<thead>
<tr>
<th>Food</th>
<th>Temperature °F (°C)</th>
<th>D-value (seconds)</th>
<th>D-value (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish Sticks</td>
<td>140 (60)</td>
<td>942.0</td>
<td>(15.7)</td>
</tr>
<tr>
<td></td>
<td>150 (65.6)</td>
<td>138.0</td>
<td>(2.3)</td>
</tr>
<tr>
<td></td>
<td>160 (71.1)</td>
<td>21.0</td>
<td>(0.35)</td>
</tr>
<tr>
<td></td>
<td>165 (73.9)</td>
<td>7.8</td>
<td>(0.13)</td>
</tr>
<tr>
<td>Tuna Pie</td>
<td>140 (60)</td>
<td>675.0</td>
<td>(11.25)</td>
</tr>
<tr>
<td></td>
<td>150 (65.6)</td>
<td>114.0</td>
<td>(1.9)</td>
</tr>
<tr>
<td></td>
<td>160 (71.1)</td>
<td>16.8</td>
<td>(0.28)</td>
</tr>
<tr>
<td></td>
<td>165 (73.9)</td>
<td>4.2</td>
<td>(0.07)</td>
</tr>
</tbody>
</table>

* Adapted from Mitscherlich and Marth (1984).
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.
- Low infective dose = 100 to 1,000 cells in a portion of food.
- Vegetative cells killed by cooking / pasteurization.

Listeria monocytogenes – Characteristics

Bacterial Characteristics

Listeria monocytogenes is a gram-positive, short rod that is motile at 6 to 77°F (20 to 25°C). 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. Animals and people can carry Listeria on their bodies without becoming ill.

Growth

Listeria monocytogenes grows from 29.3 to 113°F (-1.5 to 45°C) 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 (4°C) (Rosenow et al., 1987). Table 4-17 indicates the predicted generation times for L. monocytogenes in foods.

Table 4-17
Predicted Generation Times for 1 Multiplication of Listeria monocytogenes in Foods*

<table>
<thead>
<tr>
<th>Temperature, °F (°C)</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>32 (0)</td>
<td>16 days</td>
</tr>
<tr>
<td>35 (1.7)</td>
<td>4 days</td>
</tr>
<tr>
<td>41 (5)</td>
<td>1.2 days</td>
</tr>
<tr>
<td>45 (7.2)</td>
<td>16.7 hours</td>
</tr>
<tr>
<td>50 (10.0)</td>
<td>9.8 hours</td>
</tr>
<tr>
<td>55 (12.8)</td>
<td>6.0 hours</td>
</tr>
<tr>
<td>60 (15.6)</td>
<td>4.5 hours</td>
</tr>
<tr>
<td>65 (18.3)</td>
<td>3.4 hours</td>
</tr>
<tr>
<td>70 (21.1)</td>
<td>2.6 hours</td>
</tr>
<tr>
<td>75 (23.9)</td>
<td>2.1 hours</td>
</tr>
<tr>
<td>80 (26.7)</td>
<td>1.7 hours</td>
</tr>
<tr>
<td>85 (29.4)</td>
<td>1.4 hours</td>
</tr>
<tr>
<td>90 (32.2) to 100 (37.8)</td>
<td>1 hour</td>
</tr>
</tbody>
</table>

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

People at Risk

Most infections in people result from eating contaminated foods. Most people are not at increased risk for listeriosis. However, there are some people who are considered at risk because they are more susceptible to listeriosis. People at risk include pregnant women and their unborn babies and newborns; older adults; and people with weakened immune systems caused by cancer treatments, AIDS, diabetes, kidney disease, etc.

Hormonal changes during pregnancy have an effect on the mother’s immune system that lead to an increased susceptibility to listeriosis in the mother. According to the CDC, pregnant women are about 20 times more likely than other healthy adults to get listeriosis.

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

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 that 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. Listeriosis can be transmitted to the fetus through the placenta, even if the mother is not showing signs of illness. This can lead to premature delivery, miscarriage, stillbirth, or other serious problems for newborns. Surviving infants often contract meningitis. These newborn infants, whose mothers had listeriosis, have respiratory problems, refusal to swallow, vomiting, and nodules in the throat or on the back.

Infective Dose

The infective dose is not known at this time, but is probably 100 to 1,000 total microorganisms in susceptible persons. Taking antacids may even make seemingly healthy individuals more susceptible. (FDA, 1993).

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

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.

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. L. 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 L. monocytogenes. This finding indicates that other opened packages of food can become cross-contaminated if L. monocytogenes is present in the surrounding environment.

The estimated annual incidence of this illness in the United States is 2,500 cases, resulting in 500 fatalities (Mead et al., 1999).

In the winter of 1998-1999, a nation-wide outbreak of listeriosis in the United States occurred when it was discovered that processed meat and poultry products of a nationally recognized food production company were contaminated with Listeria monocytogenes. Product recalls were extensive throughout the United States. Poor sanitation procedures and product recontamination during packaging at a large processing facility are suspected as contributing factors to this outbreak.

References:
Anon. 1985 Listeriosis transmitted by contaminated Jalisco-brand cheese. California Morbidity. 46.
FDA, 1993. HACCP. Regulatory Food Applications in Retail Food Establishments. Dept. of Health and Human Services. Division of Human Resource Development, HFC-60; Rockville, MD.
Listeria monocytogenes – Process Hazard
Analysis and Critical Controls

Transmission
Listeria monocytogenes is a pathogenic bacteria that has been known to cause illness in cattle and sheep for sometime. Although human cases had been reported since 1930, the first documented human cases of listeriosis, traced directly to food, occurred in 1981,

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 recontamination of cured meat products (deli meats and frankfurters). A major problem with this organism is recontamination after heating. 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. Use and consume only pasteurized milk and dairy products.
2. Store products at 41°F (5°C) or less and consume within 7 days after receipt.
3. Reheat refrigerated or frozen products until all parts of the food reach 165°F (73.9°C) for 15 seconds.
4. People at risk (immune-compromised individuals and pregnant women) should not consume uncooked vegetables and salads and unheated deli meats and frankfurters.
5. Wash raw fruits and vegetables thoroughly.
6. Observe and abide by all expiration dates for perishable items that are precooked or ready-to-eat.
7. Wash hands after handling raw foods.
8. Wash and sanitize food preparation utensils after being used to prepare raw food.
9. To ensure destruction of this pathogen, food must be heated adequately. The following D values are recommended for the destruction of Listeria monocytogenes. (See Table 4-18.)

<table>
<thead>
<tr>
<th>Temperature °F (°C)</th>
<th>Time (1D) (minutes)</th>
<th>Time (4D) (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>130 (54.4)</td>
<td>21.95</td>
<td>87.80</td>
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<tr>
<td>140 (60)</td>
<td>2.85</td>
<td>11.40</td>
</tr>
<tr>
<td>150 (65.6)</td>
<td>0.37</td>
<td>1.48</td>
</tr>
<tr>
<td>160 (71.1)</td>
<td>0.047</td>
<td>0.19</td>
</tr>
</tbody>
</table>

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.

References:
CHARACTERISTICS OF SHIGELLA SPP.
- Grows with and without air.
- Grows between 43 and 116°F.
- Survives freezing temperatures.
- Source is infected people.
- Spread by fecal transmission on fingertips.
- Vegetative cells multiply in intestinal tract to cause illness (symptoms include bloody diarrhea, abdominal cramps, and fever).
- Low infective dose: 10 to 100 microorganisms in a portion of food.
- Vegetative cells killed by cooking / pasteurization.

Shigella spp. – Characteristics

Bacterial Characteristics
Shigella spp. are facultative anaerobic, nonmotile, gram-negative rods that do not ferment lactose. There are 4 known species of Shigella: Shigella dysenteriae, Shigella flexneri, Shigella boydii, and Shigella sonnei. All four species cause dysentery in humans, with the most severe illness caused by S. dysenteriae. Shigella sonnei is the leading cause of foodborne shigellosis.

Source
Man and monkeys are the only important sources. Shigella may persist in the human intestinal tract for months. Sources of incidents of shigellosis have been traced to asymptomatic carriers or people who are recovering from the disease. The disease is spread through fecal-oral transmission. Most incidents have been reported from day care centers, institutions, and the urban poor population. Shigella spp. can be transmitted in food and water, and from personal contact.

Growth
The temperature range for growth of Shigella spp. is between 43 to 116.8°F (6.1 to 47°C). The pH range for growth is 5.5 to 9.0 (depending on type) and do not survive well below pH 4.5. It has been shown to survive freezing temperatures -4°F (-20°C) for over 30 days. Shigella spp. have also been shown to survive in dilute salt solutions.

Infected Dose
Human volunteer studies indicate that ingestion of as few as 10 to 100 microorganisms can produce illness. (FDA, 1993).

Symptoms
The incubation period for shigellosis is 12 hours to 4 days after ingestion of the microorganism. Symptoms can be a mild diarrhea with no fever to a severe form of illness with frequent diarrhea, sometimes containing blood and mucus. Individuals may experience fever, abdominal cramps, and severe fluid losses. Complications include: mucosal ulceration, rectal bleeding, dehydration, reactive arthritis, and kidney disorders.

Severity of this illness depends on the type of Shigella spp., the general nutritional and immunological status of individuals, and their age. Young children and infants are most severely affected and may have fevers cause convulsions. The symptoms last for about 4 days in mild cases, but severe cases can continue for 10 to 14 days. During the acute stages of the illness, large numbers of the microorganism are passed in the feces. Infected individuals can remain as carriers of Shigella for months or longer after their illness has subsided.

Stool cultures are necessary for accurate identification of this microorganism.

Incidence
There is an estimated incidence of 450,000 cases of shigellosis each year in the United States. Of this number, 90,000 cases resulting in 14 deaths are estimated to be foodborne (Mead et al., 1999).

In 1985, a large outbreak of shigellosis occurred in Midland-Odessa, Texas, involving as many as 5,000 persons. Shigella sonnei in chopped, bagged lettuce prepared in a central location for a Mexican restaurant chain was determined to be the source of this outbreak. FDA research also determined that S. sonnei in the lettuce, which remained fresh and appeared edible, survived refrigeration temperatures (FDA, 1993).

During 1985 to 1986, several outbreaks of shigellosis on college campuses were traced to fresh vegetables on salad bars.

Another typical incident occurred in the fall of 1988 and was related to the consumption of cold food items served to people traveling on a commercial airline. The outbreak was initially recognized following identification of shigellosis among members of a professional football team. Consumption of sandwiches and salads prepared in an in-flight kitchen was associated with the outbreak. Eight of 80 food handlers from the in-flight kitchen reported having had a diarrheal illness with onset during or shortly before the outbreak. These food handlers were tested and 3 were found to be carriers of Shigella sonnei. The findings suggested contamination of cold food items (i.e., salads, sandwiches) by food handlers with Shigella Sonnei. If the types of meals associated with confirmed shigellosis were uniformly contaminated during the outbreak period, it is estimated that as many as 7,000 illnesses may have occurred.

In 1990 an outbreak of shigellosis in U.S. troops of Operation Dessert Shield was traced to fresh produce. In 1994 outbreaks of shigellosis in Norway and the United Kingdom were found to be due to iceberg lettuce imported from Spain. Green onions imported from Mexico were also implicated in outbreaks of shigellosis in the Midwest of the U.S. in the summer of 1994.

In August 1998, outbreaks of shigellosis occurred in Minnesota, California, Massachusetts, Florida, and Canada. Epidemiologic investigations revealed that chopped curly parsley was the vehicle of infection. In all outbreaks, S. sonnei was isolated from patients' stools.

Food Analysis
Methods of analysis and identification of Shigella in food are difficult. A genetic probe to the virulence plasmid has been developed by the FDA to aid in analysis and verification.
References:

FDA, 1993. HACCP. Regulatory Food Applications in Retail Food Establishments. Dept. of Health and Human Services. Division of Human Resource Development, HFC-60; Rockville, MD.


Shigella spp. – Process Hazard Analysis and Critical Controls

Transmission

*Shigella* spp. can be transmitted in both food and water. Water supplies contaminated with raw sewage have been implicated in outbreaks of shigellosis due to *S. boydii*, *S. flexneri*, and *S. dysenteriae*. Foodborne outbreaks (*S. sonnei*) are usually traced to food handlers who are ill or have been ill with the disease and do not properly wash their hands before serving and preparing food. An incident in Texas a few years ago implicated lettuce in the transmission of *Shigella sonnei*. The lettuce may have been irrigated with contaminated sewage water or it could have become contaminated by a worker defecating in the field.

Salad products such as potato, tuna, and shrimp salad were vehicles of transmission for *Shigella* spp. in foodborne outbreaks of shigellosis reported to the Centers for Disease Control and Prevention. Many of these products were prepared by carriers who did not wash their hands properly after defecating. The food products were also allowed to remain at temperatures that allowed the microorganism to survive and grow.

Control

The following controls should be used to prevent shigellosis:

1. Food handlers must be taught the importance and use of proper personal hygiene. They must use the two-step hand washing method, which utilizes a fingernail brush to scrub hands and fingernails after going to the toilet.
2. Infected individuals should not handle food items. However, there can be infected workers in food operations. This situation occurs because after the worker has no symptoms of illness, he/she will still be shedding pathogens. Fingertip and hand washing is an effective control.
3. All raw fruits and vegetables should be double washed in flowing water, in clean, sanitized sinks using clean, sanitized equipment (e.g., colanders).
4. Cold food items should be prepared using adequate methods of sanitation and must be refrigerated promptly after preparation and stored at less than 41°F (5°C).
5. Other control measures include use of properly treated water, sanitary disposal of sewage, and control of flies and rodents in food production and food storage areas.
6. Cooking foods according to the time-temperature standards for the destruction for *Salmonella* spp. assures the destruction of *Shigella* spp.

References:


**Vibrio spp. – Characteristics**

**Bacterial Characteristics**

*Vibrio* spp. are either straight or curved rods that are motile by sheathed polar flagella. They are facultative anaerobes; all metabolize glucose. Most species require sodium, so they thrive in mild salt solutions. There are over 20 recognized species and at least 10 of these species can cause illness in humans. Those vibrios of current concern as foodborne pathogens are: *Vibrio cholerae*, *Vibrio vulnificus*, and *Vibrio parahaemolyticus*.

Vibrios are naturally occurring microorganisms in marine environments and can be present in large numbers in shellfish, particularly in the warmer months of the year. They may be present in fecally contaminated water supplies.

**Vibrio cholerae**

Strains of *V. cholerae* are divided into 2 groups based on the agglutination of antisera. Those types which agglutinate antisera are known as serotype O1 and those which do not agglutinate antisera are called non-O1.

The serotype O1 *V. cholerae* has been a cause of outbreaks of diarrhea all over the world and is usually due to water contaminated with fecal material. It is present in waters on the Gulf Coast of the United States. However, no major outbreaks of this disease have occurred in the U.S. since 1911. Sporadic cases have been reported from time to time suggesting that there has been possible reintroduction of this pathogen into warm coastal waters.

This *Vibrio* can survive on shellfish from 15 to 45 days. Refrigeration temperatures prolong its survival. The incubation period for this type of cholera is 6 hours to 5 days. The symptoms include mild to severe diarrhea. If diarrhea is severe, there is rapid loss of body fluids, which results in circulatory collapse and death if no therapy is given. This is often a cause of death of people in under-developed countries.

Non-O1 *V. cholerae* is also found in contaminated drinking water and in contaminated coastal waters and shellfish. The illness caused by this strain tends to be milder than the O1 strain. Its incubation period ranges from 6 hours to 3 days. Its symptoms include diarrhea that may contain blood, abdominal cramps, nausea, vomiting, and dizziness. It usually lasts about 48 hours. This type of cholera can also cause septicemia (i.e., is carried in the blood to cause infection of other organs or parts of the body) and soft-tissue infections. Approximately 1 million organisms must be ingested to cause illness (FDA, 1993).

**Vibrio vulnificus**

This microorganism is found in seawater and seafood. It is isolated more often from clams and oysters than from lobsters and crabs. It is primarily associated with serious wound infections, life-threatening septicemia, and gastroenteritis. It has been contracted by swimmers and fishermen in the coastal waters of the Eastern United States from Cape Cod to Florida, on the Gulf Coast, and on the Pacific Coast.

The populations at risk are those with a liver disorder resulting in high levels of serum iron (e.g., liver ailments and alcoholism, etc.) Any level of *V. vulnificus* poses risk of illness and death. The infective dose is less than 100 organisms (FDA, 1993).

Following the ingestion of shellfish containing *V. vulnificus*, a primary septicemia develops which often results in death. The organism can also gain entry into the body through a break in the skin (e.g., prick of a fish hook, insect bite). Wound infections require surgery and sometimes amputations. The incubation time for symptoms to develop varies from 7 hours to several days. Ingestion of this organism produces symptoms that include fever, chills, low blood pressure, nausea, vomiting, diarrhea, abdominal pain, and possible development of secondary skin infections.

Control for this microorganism includes not harvesting or consuming shellfish taken from waters containing this microorganism during the months of April through November. Shellfish should be cooked sufficiently (using the *Salmonella* 10° pasteurization standard) to inactivate this microorganism. If shellfish are eaten in the raw state, they must be certified to be free of *V. vulnificus* by the supplier.

**Vibrio parahaemolyticus**

*Vibrio parahaemolyticus* is a gram-negative facultative anaerobe (grows in the presence or absence of air). It is common in coastal seawater and is found on fish and shellfish. It requires 0.5% to 9% salt to grow (3% is most conducive). Its optimum growth temperature is 95 to 99°F (35 to 37.2°C), but has a growth range of 41 to 111°F (5 to 44°C) [Beuchat, 1982]. Growth is inhibited below pH 4.5 or above pH 11.0. The *Vibrio* is killed in 0.5% acetic acid in a few minutes. It multiplies actively at pH 7.5 to 8.5. Poaching a fish in a court bouillon (pH 3.1) is a very effective way of killing this microorganism.

Illness results from the consumption of fish, crustaceans, and mollusks contaminated with *V. parahaemolyticus*. An infective dose of more than a million organisms cause disease. (This number is lowered markedly in individuals taking antacids or if consumed with foods that have a buffering capacity.)

Symptoms include acute onset of explosive, watery diarrhea, cramping, abdominal pain, low grade fever, mild chills, nausea, vomiting, headache, and some dehydration. Incubation times for illness range from 4 to 96 hours, typically 15 to 17 hours. The organism can multiply in the gastrointestinal tract. Recovery takes 2 to 5 days. Diagnosis
of gastroenteritis caused by *V. parahaemolyticus* is made by culturing the organism from stool specimens.

**Incidence**

Between 1988 and 1997, 345 sporadic *V. parahaemolyticus* infections were reported to the Centers for Disease Control and Prevention: 59% were gastroenteritis, 34% were wound infections, 5% were septicemia and 2% were from other exposures. Forty-five percent of these patients were hospitalized for their infections, and 88% of persons with acute gastroenteritis reported having eaten raw oysters during the week before their illness occurred.

Mead et al. (1999) estimate that there are about 8000 *Vibrio* spp. foodborne illness cases in the United States each year, resulting in over 50 fatalities.

References:


FDA, 1993. HACCP. Regulatory Food Applications in Retail Food Establishments. Dept. of Health and Human Services. Division of Human Resource Development, HFC-60; Rockville, MD.


Vibrio spp. – Process Hazard Analysis and Critical Controls

Transmission

Vibrio spp. occur naturally in sea water. They adhere to plankton in the water. During the warmer months of the year, plankton grows and rises from the bottoms of lakes, bays, oceans, and estuaries. Fish and shellfish eat the plankton and also become contaminated on their surface and in their gills with Vibrio spp. Plankton growth is encouraged by sewage waste. Hence, in warmer seasons of the year or in warm climates where water may be heavily contaminated, Vibrio populations are large.

Raw fish and shellfish can contain hazardous levels of vibrios. Given warm growth temperatures and time, cells multiply and grow to disease-causing numbers. Vibrio parahaemolyticus has a generation time of 9 to 13 minutes at an optimal temperature of 95 to 99°F (35 to 37°C) [Beuchat, 1982].

Vibrio parahaemolyticus causes over 50% of the foodborne illnesses in Japan. In Japan, raw fish (sushi) is the principle vehicle for transmission. The first reported outbreak of this foodborne illness in the United States occurred in Maryland in 1971. Since then, over 50 outbreaks have occurred. Both raw and insufficiently cooked seafoods, particularly shellfish, have been responsible for illness due to consumption of seafood products that are highly contaminated with these microorganisms.

During July to September 1998, an outbreak of Vibrio parahaemolyticus infections associated with consumption of oysters and clams harvested from Long Island Sound occurred among residents of Connecticut, New Jersey, and New York. This is the first reported outbreak of V. parahaemolyticus linked to consumption of shellfish harvested from New York waters.

During July to August 1997, the largest reported outbreak in North America of culture-confirmed V. parahaemolyticus infections occurred. Illness in 209 persons was associated with eating raw oysters harvested from California, Oregon, and Washington in the U.S. and from British Columbia in Canada. One person died.

Seafood and other foods as well can become cross-contaminated on a cutting board or by putting cooked products back into raw food containers that were not properly washed and sanitized after use.

Control

Since Vibrio spp. begin to multiply at 41°F (5°C), seafood must be kept below this temperature (Beuchat, 1982). Vibrio parahaemolyticus is easier to inactivate than Salmonella. Buechat and Worthington (1976) showed that the D value for V. parahaemolyticus in tryptocase soy broth, 0.5% salt at 117°F (47°C) was 0.8 minutes. If the same test was repeated with 7.5% salt, the D value at 117°F (47°C) increased to 6.5 minutes showing the effect of a higher 

Beuchat (1973) studied the effect of pH on V. parahaemolyticus. V. parahaemolyticus in Tryptcase soy broth with 3% salt at 127.4°F (53°C) had a D value of 4 minutes at pH 7.0 and a D value of 1.5 minutes at pH 5.0.

Fish should be heated according to the Salmonella 10,000,000:1 pasteurization standard. Most outbreaks in the U.S. have been traced to raw or under-cooked oysters, shrimp, and crabmeat that remained at ambient temperatures for several hours. A large population of Vibrio spp. has been found to survive 176°F (80°C) for several minutes when shrimp and crabmeat were cooked without a lid on the container, thus allowing the cooking surface to cool.

In order to prevent transmission of Vibrio spp., foodservice establishments should:

1. Buy seafood and shellfish from suppliers who guarantee the safety of their products.
2. Heat seafood and shellfish to temperatures for times that insure destruction of Vibrio spp.
3. Store products less than 37°F (2.8°C) for safety and less than 32°F (0°C) for quality.
4. Use sanitary handling procedures. Avoid cross-contaminating cooked and raw products.
5. Train workers who handle raw seafood to wash their hands thoroughly before handling other raw or cooked products.

There is no guarantee of safety for raw seafood unless the supplier has a microbiological quality control system.

References:


Trichinella – Characteristics

Trichinella Characteristics
Trichinella are parasitic worms (nematodes), not bacteria. Trichinella exist in both the adult form (male or female) and in a larval form, which can encyst itself in an animal or human host. Two related species, Trichinella spiralis and Trichinella nativa have been found in humans and animals in North America. Trichinella nativa is found to be carried by wild animals (red foxes, wolves, and walruses) in cold climates such as Northern Canada and Alaska, while T. spiralis is found to be carried by animals and humans in more temperate zones.

Source
Trichinella species are found in virtually all warm-blooded animals. Swine and carnivorous wild animals are the reservoirs of trichinae. Swine, in temperate zones, are usually infected by consuming viable larvae of T. spiralis in scraps found in uncooked garbage and, to a lesser degree, in meat from carcasses of infected animals such as other swine, dogs, cats, rats, mice, bears, foxes, and other carnivorous wildlife in the farm area.

Incidence
In 1940, health departments reported an average of 400 cases of trichinellosis (primarily due to T. spiralis) and 10 to 15 deaths each year. The incidence of this disease has declined since that time due to education of pork producers and consumers. Currently it is estimated that there are about 52 cases of trichinosis each year in the United States. (Mead et al., 1999).

The proportion of cases associated with heating contaminated commercial pork has declined since 1975, most likely due to laws prohibiting feeding offal to hogs, the increased use of home freezers and the practice of thoroughly cooking pork. As domestic swine-associated cases have decreased, the proportion of cases associated with eating wild game have increased.

Native Arctic peoples in Northern Canada and Alaska develop trichinosis from the consumption of raw or inadequately cooked walrus and bear meat that is infected with trichinae (T. nativa). Human infection occurs only when the meat of host animals is eaten raw or in an undercooked state.

Symptoms
After the item (raw or undercooked meat item) containing the trichinae is consumed, the larvae are released into the intestinal tract during digestion and invade the mucous membranes of the intestine where they develop into adults. Fertilized females produce many larvae, which travel through the blood and lymph to skeletal muscle where they form cysts.

The incubation period for the first symptoms to develop varies from a day or 2 to several weeks. Symptoms include nausea, vomiting, diarrhea, sweating, abdominal cramps, and loss of appetite. These symptoms are sometimes confused with other foodborne illnesses. Later symptoms, which result from encystment of larvae in muscle include headaches, fevers, chills, cough, eye swelling, aching joints and muscle pains, itchy skin, diarrhea or constipation. If the infection is heavy, patients may experience difficulty in coordinating movements, and have heart and breathing problems. In severe cases, death can occur.

Abdominal symptoms can occur 1 to 2 days after infection. Further symptoms usually start 2 to 8 weeks after eating contaminated meat. Symptoms range from mild to severe, depending on the number of infectious worms consumed in meat.

OUTBREAK EXAMPLE. The following example appeared in MMWR 40(4):57-60, 1991.

Epidemiologic Notes and Reports Trichinella spiralis infection--United States, 1990. From July 21 through September 3, 1990, 90 people of 250 persons who attended or ate food taken from a wedding in Des Moines on July 14 developed trichinosis. Most of the people who attended the wedding had immigrated to the U.S. since 1975 from Southeast Asian countries. Of those who became ill, 52 were treated by physicians, and one person was hospitalized.

Detailed case histories were obtained from 39 ill and 13 well persons who attended the wedding. Of the 39 ill persons, 34 had eaten uncooked pork sausage, compared with 4 of the 13 well persons. No other foods were associated with the illness. The sausage had been prepared from 120 pounds of commercially purchased pork and was served uncooked as is customary for that food item in Southeast Asian Cultures.

The meat could not be traced back to the source farm because the meat packing company that supplied the pork slaughters 14,000 to 15,000 hogs a day from hundreds of farms and the exact date the hogs were slaughtered was unknown.

Only 4 of 107 persons who attended the wedding and were interviewed knew about trichinosis, or about the potential hazards of eating undercooked pork. The Iowa Refugee Health Program and Iowa Department of Health, prepared a brief information sheet describing trichinosis and ways to avoid infection and translated this information for members of the Southeast Asian community in Iowa.

Another outbreak in which 15 people became ill with diagnosed trichinellosis occurred in Virginia in November and December 1990. Epidemiologic evidence determined that

1 Trichinellosis and trichinosis are synonymous and refer to illness / disease caused by trichinae.
these persons consumed uncooked or undercooked pork sausage.

**Outbreak Example.** The following example appeared in MMWR 45(10):205-206, 1996.

**Outbreak of Trichinellosis associated with eating cougar jerky -- Idaho, 1995.** During the second week of January 1995, a man shot and killed a cougar near Elk City, Idaho. During January 15 through 18, he prepared jerky from the cougar meat by first soaking the meat in a brine solution made from table salt, then smoking the meat. However, he later reported that the smoker never become more than warm. During the next 4 weeks, he distributed the meat to 14 other persons, all of whom ate the meat within days to 1 month of receipt.

On January 26, the man became ill with symptoms of fever, myalgia, arthralgia, facial swelling and fatigue. Medical tests confirmed the presence of *Trichinella* spp. antibody. *Trichinella* spp. larvae were also identified in specimens of jerky and fresh frozen cougar muscle.

During March 3 to April 10, the 14 people who had received jerky were interviewed. Of these 14, 9 people were identified as having trichinellosis. The patients were treated with mebendazole and were educated on trichinellosis prevention.

**References:**
Trichinae – Process Hazard Analysis and Critical Controls

Transmission

Animals such as swine, bears, and even horses become infected by eating garbage, contaminated food, or another infected animal. Seals and walruses can also become infected. If parasites such as trichinae are present in the muscles of a slaughtered infected animal and the meat (muscle) is not sufficiently heated, the trichinae can pass on to another host.

The FDA Food Code recommends cooking large solid cuts of pork (roasts) until every part of the meat reaches a temperature of:

- $130^\circ F$ ($54.4^\circ C$) for 112 minutes or
- $135^\circ F$ ($57.2^\circ C$) for 36 minutes or
- $140^\circ F$ ($60^\circ C$) for 12 minutes or
- $145^\circ F$ ($63^\circ C$) for 4 minutes.

Small solid cuts of pork (e.g., pork chops, pork steak) must be cooked to $145^\circ F$ ($63^\circ C$) for 15 seconds, according to the FDA Food Code.

Although ground beef is generally considered to be pure beef, it may become adulterated with ground pork, either by contamination from an uncleaned, common meat grinder that was previously used to grind pork or by the intentional mixing of beef and pork. The FDA Food Code recommends that ground (comminuted) meats (including ground pork) must reach $155^\circ F$ ($68^\circ C$) for 15 seconds; or $150^\circ F$ ($66^\circ C$) for 1 minute; or $145^\circ F$ ($63^\circ C$) for 3 minutes. Heating ground meat products to these temperatures assures the destruction of pathogenic bacteria such as *Escherichia coli* O157:H7 and *Salmonella* spp. as well as inactivation of *Trichinella spiralis*.

In recent years, trichinosis has occurred most frequently among members of ethnic groups who enjoy eating raw pork. Recent outbreaks have occurred among new immigrants who apparently did not understand the need to cook, freeze, or otherwise treat American pork thoroughly in order to kill trichina larvae. Other recent outbreaks have been reported for Eskimos who have consumed under-cooked seal or walrus tissue.

For safety, the FDA Food Code recommends cooking small, solid cuts of pork until every part of the meat reaches a temperature of $145^\circ F$ ($63^\circ C$) for 15 seconds, and cooking ground (comminuted) pork products until the temperature reaches $155^\circ F$ ($68^\circ C$) for 15 seconds; or $150^\circ F$ ($66^\circ C$) for 1 minute; or $145^\circ F$ ($63^\circ C$) or 3 minutes. However, many consumers prefer the flavor of pork cooked to a higher well-done temperature of 170 to $180^\circ F$ ($76.7$ to $82.2^\circ C$).

Detection

The larvae of *T. spiralis* in meat can be detected by several methods, including microscopic examination of fresh or digested meat and by enzyme-lined immunosorbent assay (ELISA). While larvae detection methods are routinely used in many countries for examination of swine after slaughter, they are not used in the United States as a means of preventing infected meat from reaching the consumer. Therefore it must be assumed that all pork (and game meat) is contaminated and these raw meat products should be heated to temperatures for times that assure the destruction of trichinae. These procedures are considered more effective in preventing human infection and less expensive than is a routine examination of meat for larvae.

Trichinae Control

Research studies in 1919 (Ransom and Schwartz) showed that heating solid muscle to $137.5^\circ F$ ($58.6^\circ C$) for 15 seconds with a slow cooking process was adequate for *T. spiralis* inactivation.

Live trichinae can survive rapid cooking methods such as microwave cooking. The shorter exposure time to lethal temperatures and unevenness of heating (cold spots in products cooked in a microwave oven) enable trichinae to survive in meat. The FDA Food Code recommends that when raw animal foods are cooked in a microwave oven, the items shall be:

1. Rotated or stirred throughout or midway during cooking to compensate for uneven distribution of heat.
2. Covered to retain surface moisture.
3. Heated until all parts of the food reach a temperature of $165^\circ F$ ($76.7^\circ C$) and are allowed to remain at this temperature for 2 minutes.

USDA Inspection and Regulations

USDA inspection, as mentioned previously, does not determine the presence of trichinae. Pork products purchased at a local supermarket or butcher shop have been reported to be responsible for approximately 2/3 of the reported cases of pork-associated trichinosis. Recent data on the prevalence of *T. spiralis* in commercially slaughtered swine indicate that approximately 1 in 1,000 carcasses is infected. Even though laws prohibit the feeding of garbage to swine, it is difficult to enforce this practice and control swine-feeding practices. Hogs are carnivorous and eat rats found in their environment.

In order to ensure the destruction of *T. spiralis* in raw pork products, the USDA has developed freezing recommendations for commercial producers of undercooked pork items (e.g. sausages, pepperoni). (It should be noted that while these freezing temperatures inactivate *T. spiralis*, freezing does not provide an adequate destruction of pathogenic bacteria and viruses.)

The following are USDA freezing regulations for destruction of trichinae in pork products (9CFR 381.10):
Table 4-19

For pieces of meat 6 inches or less in thickness arranged in layers not exceeding 6 inches in depth

<table>
<thead>
<tr>
<th>Temperature °F (°C)</th>
<th>Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5(-15)</td>
<td>20</td>
</tr>
<tr>
<td>-10(-23.3)</td>
<td>10</td>
</tr>
<tr>
<td>-20(-28.9)</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 4-20

For pork in containers or in pieces or layers between 6 and 27 inches thick

<table>
<thead>
<tr>
<th>Temperature °F (°C)</th>
<th>Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (-15)</td>
<td>30</td>
</tr>
<tr>
<td>-10 (-23.3)</td>
<td>20</td>
</tr>
<tr>
<td>-20 (-28.9)</td>
<td>12</td>
</tr>
</tbody>
</table>

It should also be noted that the freezing times and temperatures given for inactivating *T. spiralis* in pork are not effective for certain strains of trichinae found in wild game meat (e.g., *T. nativa*).

**Other Parasites in Meat**

At least a dozen or more other foodborne parasites are transmitted to man from meat, fish, and shellfish. These include tapeworms (cestodes), flukes (trematodes), roundworms (nematodes), and the parasitic protozoa, *Toxoplasma gondii*, *Sarcocystis hominis*, and *Sarcocystis suihominis*. Heating meat products to temperatures for times necessary for the destruction of trichinae is sufficient to inactivate these parasites.

References:

CDC. 2005 Fact Sheet. Trichinellosis.
http://www.cdc.gov/ncidod/dpd/parasites/trichinosis/factsht_trichinosis.htm


Hepatitis A Virus – Characteristics

**Viral Characteristics**
There are several types of hepatitis. Hepatitis A and hepatitis E (also known as non A, non B hepatitis) are mainly transmitted through the fecal-oral route, while hepatitis B, C, and D are spread through blood or other body fluids (serum hepatitis). Hepatitis A virus causes the illness commonly known as infectious hepatitis.

Hepatitis A virus (HAV) is very small. Purified particles are 27 nanometers in diameter and possess single-stranded RNA. It is quite difficult to culture viruses and knowledge gained about hepatitis A virus has been achieved by studying the isolated virus particles from the feces of infected humans.

Viruses, although incapable of multiplying in food, may remain viable in food for weeks and in frozen food for months. (Lyn, 1966).

Millard et al. (1987) studied the destruction of hepatitis A virus in experimentally contaminated cockles (bivalve mollusks). Their study indicated that the virus was inactivated by 1 minute of immersion in boiling water or by 1.5 minutes of steaming, at which an internal temperature of 185 to 190°F (85 to 90°C) was achieved in the shellfish. These times and temperatures seem to indicate that the virus is resistant to inactivation at milk pasteurization temperatures.

**Source**
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.

**Infective Dose**
The infective dose is unknown, but is probably very low, less than 100 virus particles.

**Symptoms**
Hepatitis A (HAV) is highly contagious. The incubation period is from 10 to 50 days. The average incubation time is 28 to 30 days.

People are very infective from the latter half of the incubation period to a few days after the onset of jaundice. During this time they pass the virus in their feces, urine, and other body fluids. This means that infectious individuals, such as food handlers or children, can spread the disease well before they are even aware of it.

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, 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 a 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 adults, approximately 22 percent will be hospitalized. In some cases the illness can be severely disabling and 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. Convalescence is usually prolonged. An estimated 100 deaths occur in the U.S. each year from hepatitis A.

**Immunity**
Prior infection with hepatitis A provides a lifetime immunity against a second attack. A blood test can determine if an individual has had hepatitis A in the past. An injection of immune globulin can be used to provide short-term immunity (3 to 6 months). Vaccines for hepatitis A have also been developed to provide longer-term immunity.

**Outbreaks**
Hepatitis A virus is the seventh most commonly reported infectious disease in the United States. In 1999, approximately 17,000 cases of HAV were reported in the U.S. Mead et al. (1999) estimate that there are over 83,000 cases of HAV. Of this number, only 4, 170 cases resulting in 4 deaths are estimated to be due to foodborne transmission. Many cases are due to polluted water and direct transmission from carriers of the virus.

Outbreaks occur in institutions, day care centers, schools, low-cost housing projects, rural areas, and in military forces during a war.

An early record of food-associated hepatitis in the U.S. dates back to 1946 when infectious hepatitis was isolated in food eaten at a fraternity house. The probable cause of this episode was a food handler who contaminated other foods during their preparation. Table 4-21 indicates some typical documented infectious hepatitis A incidents.
<table>
<thead>
<tr>
<th>Year</th>
<th>Vehicle</th>
<th>Site of Outbreak</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>Imported lettuce</td>
<td>Louisville, Kentucky</td>
</tr>
<tr>
<td>1988</td>
<td>Ice slush beverage</td>
<td>Alaska</td>
</tr>
<tr>
<td></td>
<td>Iced Tea</td>
<td>North Carolina restaurant</td>
</tr>
<tr>
<td></td>
<td>Raw oysters</td>
<td>Non approved bed, Florida</td>
</tr>
<tr>
<td>1990</td>
<td>Frozen strawberries</td>
<td>North Georgia</td>
</tr>
<tr>
<td></td>
<td>Frozen strawberries</td>
<td>Montana</td>
</tr>
<tr>
<td></td>
<td>Shellfish</td>
<td>Baltimore, Maryland</td>
</tr>
<tr>
<td>1997</td>
<td>Frozen strawberries</td>
<td>School Lunch Programs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>in Michigan and Maine</td>
</tr>
</tbody>
</table>

**OUTBREAK EXAMPLES.** The following examples appeared in MMWR 42(27):526-529, 1993.

**Foodborne Hepatitis A -- Missouri, Wisconsin, and Alaska, 1990-1992.**

**Missouri.** On November 26, 1990, hepatitis A was diagnosed in an employee of a restaurant in Cass County, Missouri. The employee's duties involved washing pots and pans in the restaurant. From December 7, 1990 through January 9, 1991, hepatitis A was diagnosed in 110 persons, including four waitresses, who had eaten at the restaurant. Two persons died as a result of severe illness due to hepatitis.

It was determined that case patients were most likely to have eaten salads and sandwiches containing lettuce. The index case patient (the dishwasher) also reported that he occasionally helped unpack fresh produce and prepare lettuce for salads.

**Milwaukee, Wisconsin.** On April 10, 1991, a food handler who worked at two sandwich shops was diagnosed with hepatitis A. From April 17 through May 29, hepatitis A was diagnosed in 230 persons. Because 228 of the 230 case-patients ate exclusively at one of the two shops and because no prepared food was shared between them, food was considered to have been contaminated independently at each sandwich site. (The source of contamination was most likely the infected food handler.)

**Alaska.** On May 4, 1992, a food handler who routinely prepared uncooked sandwiches at a fast-food restaurant in Juneau, Alaska, had onset of nausea, vomiting, and diarrhea. Although his supervisor instructed him not to handle food, he was allowed to continue work. On May 8, he sought medical attention and was jaundiced. On May 18 he tested positive for hepatitis A. The food handler reported that his hygiene was good, and this was confirmed by his supervisor and co-workers.

However, from June 1 through June 11, there were 11 cases of acute hepatitis A diagnosed in residents or visitors to Juneau. All of these individuals had eaten once during May 4-8 at the fast-food restaurant where the infected food handler worked.

Factors that are essential in the prevention and control of foodborne hepatitis A include accurate assessment of the hygienic status of food handlers; identification of food handlers and other restaurant employees with hepatitis A; and rapid diagnosis and reporting of cases in food handlers.

**References:**


Hepatitis A Virus – Process Hazard Analysis and Critical Controls

Hepatitis A Virus Transmission
Hepatitis A virus is transmitted by the fecal-oral route, through close person-to-person contact, or by ingesting contaminated food and water. The foodborne sources of the illness are generally associated with raw foods or cooked foods that become contaminated after heat processing.

Infection has been shown to be spread by:

- some form of fecal-oral close personal contact with someone infected with hepatitis A.
- eating foods contaminated by the hands of infected food handlers.
- contact with the fecal material of infected children (who do not usually show symptoms) who can then infect non-immune children or adults at home or in child-care centers.
- ingesting raw or undercooked shellfish (oysters, clams, mussels etc) taken from waters contaminated with hepatitis A virus.
- ingesting contaminated food or water during travel to underdeveloped areas.

Food handlers transmit hepatitis A virus when they are either acutely ill or are carriers. They excrete the virus in their feces and urine and do not use adequate hand washing procedures before preparing or handling food. Other means of transmission include food or water contaminated with raw sewage. This type of contamination can occur in foodservice establishments, when plumbing is faulty and sewage leaks out of pipes or backs up in sinks or on floors and then gets into the food.

Hepatitis A Virus Control
In order to prevent the transmission of hepatitis A virus foodservice establishments should:

1. Train and mandate foodservice workers to use the two-step hand washing procedure that uses a fingernail brush and to scrub hands and fingernails after going to the toilet.
2. Double wash fruits and vegetables that will not be cooked in a sanitized sink, using a safe water supply.

The water dilutes the viruses to a level low enough that the hazard is controlled.
3. Buy seafood from suppliers who certify that the seafood or shellfish was taken from safe waters. If the seafood or shellfish is not obtained from a certified source, it must be heated so that an internal temperature of 185 to 190°F (85 to 87.8°C) is reached to insure the destruction of hepatitis A virus.
4. Use only clean sanitized equipment and utensils to mix food and food products.
5. Discourage ill personnel from working with food.
6. Encourage people at risk of contacting hepatitis A to be vaccinated.

References:
Noroviruses (Norwalk-like Viruses) – Characteristics

Viral Characteristics
Noroviruses (Caliciviruses) are small, round-structured viruses, 25 to 35 nanometers in diameter. A virus of this type was first isolated in 1972 in Norwalk, Ohio. Since that time, these small round-structured viruses have been identified as a cause of gastroenteritis in many countries throughout the world.

Noroviruses are difficult if not impossible to culture. Most of the data collected about these viruses have come from stool samples and food or water samples from which they were isolated.

Source
Humans are the source of these viruses by way of fecal-oral transmission. These viruses can be spread easily from person to person. They may be spread in day care centers through changing of diapers and inadequate methods of washing hands after touching fecal material and other body fluids by both children and staff members. They are often found in water containing raw sewage.

 Infective Dose
The amount of noroviruses needed to cause illness is unknown, but probably very low, perhaps 1-10 virus particles.

Symptoms
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 and last from 24 to 60 hours. The illness is rarely fatal. Once the infected person has recovered, the disease may be passed to others for up to 7 days after onset. In this way, the illness often spreads quite easily through schools, camps, and families.

Susceptibility and Immunity
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).

Incidence
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
The illness causes extreme discomfort but is rarely fatal. Noroviruses are very infective. 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. Another incident involved a sick salad preparation worker who 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.

In another outbreak, 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. In still another reported incident, a salad worker who did not thoroughly wash her hands after cleaning up a child’s vomit infected hundreds of customers.

Mead et al. (1999) estimates that annual incidence of foodborne illness due to noroviruses is over 9 million cases. Noroviruses cause more foodborne illness than all bacterial pathogens combined.

References:
Centers for Disease Control. 2001 Norwalk-like viruses - Public health consequences and outbreak management. MMWR 50 / No. RR-9: 1-17.
FDA, 1993. HACCP. Regulatory Food Applications in Retail Food Establishments. Dept. of Health and Human Services. Division of Human Resource Development, HFC-60; Rockville, MD.
Noroviruses – Process Hazard Analysis and Critical Controls

Transmission
Noroviruses (Norwalk-like viruses) do not multiply in food but may be carried by air, water, or uncooked food to a human host in whom the virus will multiply. Heat destroys the virus. These small round structured viruses are recognized as an important cause of water-borne illness. Under naturally occurring conditions in a contaminated water supply, routine chlorination alone will not inactivate these viruses. Keswick et al. (1985) found that 3.75 ppm chlorine, which is used in most water treatment facilities, was not effective in inactivating noroviruses. If drinking or recreational water is suspected as being an outbreak source, high-level chlorination (e.g., 10 ppm or 10 mg/L for >30 minutes) 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. The exact thermal inactivation temperature of the virus is unknown at this time. However, milk pasteurization temperatures [161°F (72.2°C) for 15 seconds] seem to be effective. This means that if food products are heated to this temperature or above after contamination, the virus will be inactivated.

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.

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 fingers 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 160°F (71.1°C) or above for over 15 seconds.
5. Clean, sanitized equipment should be used to mix, serve, and store food.

Because the virus continues to be present in the stool for as long as 2 to 3 weeks after the person feels better, strict hand washing after using the bathroom and before handling food items is important in preventing the spread of this virus. Food that may have been contaminated by an ill person should be disposed of properly.

Generally, people can decrease their chance of becoming ill with a norovirus by following these preventive steps:

- Frequently washing hands, especially after using the toilet and changing diapers and before eating or preparing food.
- Carefully washing fruits and vegetables, and steaming oysters before eating them.
- Thoroughly cleaning and disinfecting contaminated surfaces immediately after an episode of illness by using a bleach-based household cleaner.
- Immediately removing and washing clothing or linens that may be contaminated with virus after an episode of illness (using hot water and soap).
- Flushing or discarding any vomitus and/or stool in the toilet and making sure that the surrounding area is kept clean and sanitized.

References:
Centers for Disease Control. 2001 Norwalk-like viruses - Public health consequences and outbreak management. MMWR 50 / No. RR-9: 1-17.
**Staphylococcus aureus — Characteristics**

**Bacterial Characteristics**

Staphylococci are characterized by a group of cocci (round cells) combined in grape-like clusters. (Cells may also exist in pairs or in short chains.) The cocci are tiny, less than 1 micron in diameter. Staphylococci do not form spores. Over 20 strains of Staphylococci are known. Strains that are coagulase-positive are known to cause foodborne illnesses due to the toxins they produce. Of these strains, coagulase-positive *Staphylococcus aureus* is the most common source of toxin production.

**Source**

*Staphylococcus aureus* is commonly found in the throat, on hair, in feces, and on the skin of humans and animals throughout the world. *Staphylococcus aureus* can be found in 30 to 50% of the noses and on the hands of 14 to 40% of healthy people. This microorganism is usually present in abscesses or sores and in bandages that cover sores. Healthy individuals are capable of spreading this pathogenic bacteria everyday. It may be present in raw (unpasteurized) milk. Cells of *S. aureus* may be found in dust and soil, on clothes, floors, and walls and in frozen foods.

When a large number of *S. aureus* is present and grows in food, toxins are produced. Ingestion of foods containing these toxins causes illness. Vegetative cells are destroyed by most cooking methods. However, toxins are heat stable and are not destroyed when products are boiled [212°F (100°C)] for 25 minutes. The most effective way of preventing foodborne illness caused by *S. aureus* is to prevent its bacterial multiplication and subsequent toxin production in food.

**Growth Conditions**

**Atmosphere.** *Staphylococcus aureus* grows in the presence or absence of air, although growth is more rapid in the presence of air. It can grow on or within food.

**Nutrients.** It requires an adequate supply of high quality protein and is capable of rapid multiplication in foods such as meat, milk, eggs, poultry, or custard.

Many strains are salt tolerant and grow well in media of moderately high (10%) salt concentrations. Others multiply in brine of very high concentration of NaCl (up to 20%). Pereira et al. (1982) found that no enterotoxins were produced in salt concentrations above 10%. Staphylococci are tolerant of nitrates, and can contaminate cured meats during and after processing. Staphylococci are also tolerant of dissolved sugar and grow well in cream filled pastries.

**Temperature.** The temperature range within which *S. aureus* may grow is affected by strain, number of cells, and the food or media. Temperatures between 43.8 to 122°F (6.5 to 50°C) may be expected to support growth of *S. aureus* (Halpin-Dohnalek and Marth, 1989b). Optimum growth occurs between 86 to 104°F (30 to 40°C). Under optimal conditions for growth, *S. aureus* double their numbers every 19 minutes (in skim milk) after a 1-hour lag (Halpin-Dohnalek and Marth, 1989a).

Demchick et al. (1982) found that the organisms survived freeze-thaw cycling and that destruction did not occur unless a low pH was combined with freeze-thaw stress.

**pH.** *Staphylococcus aureus* is capable of growing in substrates of a wide range of pH, from 4.5 to 9.3 (Bergdoll, 1989). Optimal growth occurs in neutral media of pH 7.0 to 7.5. The minimal pH for growth is lower under aerobic than under anaerobic conditions.

**Water Activity (a\(_w\)).** *Staphylococcus aureus* will grow in aerobic conditions in a water activity range of 0.83 to greater than 0.99. Most authorities state that *S. aureus* does not produce toxin below 0.86 \(a_w\). However, Lee et al. (1981) found that *S. aureus* grew and produced toxin in fried bacon at 99°F (37°C) at a water activity of 0.84 (aerobically) and 0.90 (anaerobically). These microorganisms grow in liquid foods as well as on solid foods of suitable moisture content.

**Competitive microorganisms.** The growth of *S. aureus* is affected by the presence and growth of other microorganisms in the same media or environment. *S. aureus* may be detected in many raw foods. Its presence usually presents no problems because of the competitive effects of greater numbers of other microorganisms (Bergdoll, 1989). Goepfert and Kim (1975) reported that *S. aureus* was unable to grow in raw ground beef that was held at temperatures ranging from 34 to 54°F (1 to 12°C) for 14 days. Competitive effects have been found to be very complex and are affected not only by temperature, but also by the composition and pH of the media.

However, the competitive effects that result from the growth of other microorganisms in food should not be relied on as a method to control the growth *S. aureus*. Some lactic acid bacteria inhibit the growth of *S. aureus* while others stimulate its growth.

In heated foods, most competitive microorganisms have been eliminated, so that if *S. aureus* recontaminates the food and conditions are conducive to its rapid growth, the multiplication of this microorganism and its subsequent toxin production can produce a hazardous food product.

**Other factors.** Growth is slowed by compounds in some spices such as sage, marjoram, mustard, garlic, paprika, and clove. Usually the amount required it so great that this method is impractical. Root products such as radishes and horseradish have no effect on the growth of *S. aureus*.
**Toxin Production**

Several enterotoxins have been identified. These include toxins A, B, C, C2, D, and E, as well as other unidentified toxins. Enterotoxins A and D are most commonly involved in staphylococcal food intoxication outbreaks (Halpin-Dohnalek and Marth, 1989b).

Growth of *S. aureus* to a population of 100,000 (10^5) or more cells per gram of food is necessary for sufficient toxin production (slightly less than 1 microgram in contaminated food) to produce symptoms of food intoxication (FDA, 1993). This means that a population of 1,000 (10^3) per gram of food would not be hazardous.

Strain, inoculum concentration, and media affect toxin formation. Toxin production begins at 50°F (Tatini, 1973). The optimal temperature range for both growth and toxin production is 70 to 104°F (21 to 40°C). Enterotoxin production continues to 114.8°F (46°C). Under favorable growth conditions, the toxin becomes evident after 4 to 6 hours of active multiplication.

Scheusner and Harmon (1973) determined that enterotoxins A, B, C, and D could be produced in a variety of foods with pH values of 5.5 to 6.6 but not in foods below pH 5.0. Tatini et al. (1971) found that enterotoxin A was produced in milk at pH values from 4.5 to 6.5 pH.

**Symptoms of Illness**

All people are believed to be susceptible to this type of bacterial intoxication; however, intensity of symptoms may vary.

There is usually a rapid onset of symptoms, which include severe abdominal pain and cramping, nausea, diarrhea, and vomiting. They occur 30 minutes to 6 hours after ingestion of food in which *S. aureus* grew and produced enterotoxin. The illness usually lasts less than 24 hours and is rarely fatal.

Some individuals may not always demonstrate all the symptoms associated with the illness. In more severe cases, headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur. In infants and the elderly, fluid replacement may be required if there have been great fluid losses through diarrhea and vomiting. In severe cases, recovery may take three days or longer.

In a review of *S. aureus*, Halpin-Dohnalek and Marth (1989b) discussed the effect of *S. aureus* toxins and reported that the emetic effect of the toxin lies within the intestinal tract. However, the staphylococcal toxins do not act directly on intestinal cells and thus are not considered to be classic enterotoxins. Action of the staphylococcal enterotoxins generates impulses to the vomiting center of the brain. In effect then, staphylococcal toxins can be regarded as neurotoxins based on this mode of action.

**Toxic Dose**

Evenson et al. (1988) reported that 94 to 184 nanograms (144 ng. average) of enterotoxin A in chocolate milk was the amount that caused illness in children. Therefore, a toxic dose of less than 1.0 microgram in contaminated food produces symptoms of illness. This level of toxin production is reached when populations of *S. aureus* exceed 100,000 (10^5)/gram (FDA, 1993).

It is also important to note that the growth of a population of this size in food will not adversely affect its flavor and odor; hence the food is consumed because it still tastes and smells good.

**Food Analysis**

In order to detect trace amounts of staphylococcal enterotoxin in foods, the toxin must be separated from the food constituents and concentrated before it can be identified. The toxin is selectively adsorbed on ion exchange resins and then food constituents are removed from the extract leaving the enterotoxin in solution. Other more recently developed rapid methods of analysis are also being evaluated for efficacy. These methods include ELISA and Reverse Passive Latex Agglutination.

**Incidence**

Mead et al. (1999) estimate the incidence of staphylococcal food poisoning to be over 185,000 cases each year. The true incidence of staphylococcal food poisoning is unknown for a number of reasons, which include: unreported incidents; misdiagnosis of the illness, which may be symptomatically similar to other types of foodborne illness (e.g., vomiting can be caused by *Bacillus cereus* toxin); improper collection of samples for laboratory analysis; and improper laboratory examination. Many times, incidents may go unreported, due to the short duration of the illness. Unless a large number of people are involved, incidents may not be reported. Reported incidents usually involve institutions, restaurants, caterers, and cured or fermented meat processors.

The following reported incidents illustrate several characteristics of staphylococcal foodborne outbreaks. Outbreaks occur most frequently in foods that are high in protein.

**OUTBREAK EXAMPLE I**

The following example appeared in MMWR 1997 46 (50) 1189-1191.

**Outbreak of Staphylococcal Food Poisoning associated with Precooked Ham -- Florida, 1997.** On September 27, 1997, a community hospital in northeastern Florida notified health authorities that several persons were in the emergency department because of gastrointestinal illnesses suspected of being associated with a common meal. The findings of the Florida Department of Health implicated staphylococcal intoxication as the cause of illness among persons who attended a retirement party on September 26, 1997.

A case was defined as nausea and/or vomiting in a person who attended the party or consumed food served at the party and became ill within 8 hours after eating.

Of approximately 125 people who attended the party, 98 completed and returned questionnaires. A total of 18 persons had illnesses meeting the case definition. Ill persons reported nausea (94%), vomiting (89%), diarrhea (72%), weakness (67%), sweating (61%), chills (44%), fatigue (39%), myalgia (28%), headache (11%), and fever (11%). Onset of illness occurred 1 to 7 hours after eating and symptoms lasted from 2 to 72 hours. Seven persons sought medical treatment and 2 were hospitalized overnight. Illness was associated with eating ham.
One sample of leftover cooked ham and one sample of leftover rice pilaf were analyzed and found to be positive for staphylococcal enterotoxin A.

On September 25, a food preparer had purchased a 16 pound precooked packaged ham, baked it at home at 400°F for 1.5 hours, and transported it to her workplace, a large institutional kitchen, where she slice the home while it was hot on a commercial slicer. The food preparer reported she had not cuts, sores, or infected wounds on her hands. She reported that she routinely cleaned the slice in place rather than dimanteing it and cleaning it according to recommended procedures and that she did not use an approved sanitizer. All 16 pounds of sliced ham had been placed in a 14-inch by 12-inch by 3-inch plastic container that was covered with foil and stored in a walk-in cooler for 6 hours, then transported back to the preparer's home and refrigerated overnight. The ham was served cold at the party the next day. The rice pilaf was prepared the day of the party by a different person.

Although the exact source of contamination for the ham is unknown, the ham could have been contaminated by the food preparer's hands, even though there was no sign of staphylococcal infection. Only 1/3 of food handlers from whom staphylococci are isolated have symptoms consistent with an active staphylococcal infection. The ham may have been contaminated by contact with the slicer because the slicer had not been cleaned adequately. Slicing the ham when the ham was warm increased the surface area and provided a favorable temperature for replication of toxin producing organisms. In addition, placement of a large quantity of warm, salty ham in a small, tightly closed container prevented rapid cooling and extended the time during which growth and toxin production occurred.

OUTBREAK EXAMPLE II. The following example appeared in MMWR 38 (24): 417-418, 1989.

Epidemiologic Notes and Reports - Multiple Outbreaks of Staphylococcal Food Poisoning Caused by Canned Mushrooms.

Starkville, Mississippi. On February 13, 1989, 22 people became ill after eating at a university cafeteria. Symptoms included nausea, vomiting, diarrhea, and abdominal cramps. Nine people were hospitalized. Canned mushrooms served with omelets and hamburgers were associated with illness. No deficiencies in food handling were found. Staphylococcal enterotoxin type A was identified in a sample of implicated mushrooms from the omelet bar and in unopened cans from the same lot.

Queens, New York. On February 28, 1989, 48 people became ill within about 3 hours after eating lunch in a hospital employee cafeteria. One person was hospitalized. Canned mushrooms served at the salad bar were implicated. Two unopened cans of mushrooms from the same lot contained staphylococcal enterotoxin type A.

McKeesport, Pennsylvania. On April 17, 1989, 12 people became ill with gastroenteritis about 2 hours after eating lunch or dinner at a restaurant. Two people were hospitalized. Canned mushrooms consumed on pizza or with a parmigiana sauce were associated with illness. No deficiencies were found in food preparation or storage. Staphylococcal enterotoxin was found in samples of remaining mushrooms and in unopened cans from the same lot.

Philipsburg, Pennsylvania. On April 22, 1989, 20 people developed illness several hours after eating food from a takeout pizzeria. Four people were hospitalized. Only pizza served with canned mushrooms was associated with illness. Staphylococcal enterotoxin was found in a sample of mushrooms from the pizzeria and in unopened cans with the same lot number.

All cans implicated in these mushroom-associated outbreaks were large institution sized (#10) cans containing stems and pieces. The mushrooms were produced and processed in the Peoples' Republic of China and were shipped through Hong Kong. Thermal processing records indicated that the mushrooms had been adequately processed. However, evidence suggested that the staphylococcal out breaks were the result of preprocess formation of enterotoxin in fresh, blanched, or salt-brined mushrooms and subsequent survival of the enterotoxins in the thermal process. Several Class II recalls and product seizures of imported canned occurred in 1991, 1992, 1994, and 1995 because of product contamination with staphylococcal enterotoxins. As a result, canned mushrooms from the Peoples' Republic of China are now under automatic detention by the U.S. Food and Drug Administration and entry is permitted only on a lot-by-lot basis, once the product is demonstrated to be free from staphylococcal enterotoxins.

Outbreaks usually occur when a contaminated food is held at temperatures that allow S. aureus to grow and produce toxin in food. The toxin is heat stable, and reheating foods will not prevent the illness. Human carriers are usually the source of S. aureus. Carriers often do not have lesions, thus, absence of nose and hand lesions is no guarantee of safety.

References:
FDA, 1993. HACCP. Regulatory Food Applications in Retail Food Establishments. Dept. of Health and Human


**Staphylococcus aureus – Process Hazard Analysis**

**Typical Hazards**

Outbreaks of staphylococcal foodborne illness are most often associated with processed red meats, precooked poultry, meat, and fish products (especially chicken, other meat, or fish salads), egg salads, potato salad, sauces, dairy products (milk, cheese and butter), and custard or cream-filled baked products. Most outbreaks occur as the result of contamination of precooked food, often through unsanitary handling and holding the food at temperatures that allow the growth and production of toxins by *Staphylococcus aureus*.

**Critical Conditions**

In many of these food products, the protein constituent has been thoroughly cooked to kill vegetative (growing) stages of all bacteria. During and after processing and cooking, food can become contaminated with *S. aureus* by foodservice workers during mixing, slicing, or other handling procedures. In turn, the contaminated food, such as sliced or cubed ham and chicken, may be allowed to remain at room temperature for hours. It may be held in warming tables or cabinets at lower than approved hot holding temperatures, or it may be stored in large containers in refrigerators for long periods of time.

Cured meat products such as ham contain high levels of salt and some nitrates, which inhibit the growth of other bacteria but allow the growth of *S. aureus* under optimum growth temperatures and pH.

Items frequently indicted are menu items that have received a large number of handling procedures. They have a better chance of becoming contaminated with the bacteria clinging to human hands and unsanitary equipment. Hazardous items are those protein products which are cut, sliced, and cubed, then placed in a sauce and not sufficiently reheated; items which are allowed to cool slowly and therefore remain in the danger zone of bacterial growth for long periods of time; and items which are "warmed over" several times without ever getting hot enough for the contaminants to be inactivated.

Small numbers of *S. aureus* are to be expected in foods that have been exposed to or handled by food handlers. The only way to prevent the staphylococcal intoxication is to control bacterial multiplication.

Time is also critical. If foods contaminated with *S. aureus* are given time in proper growth conditions, the number of organisms will increase and toxin will be produced.

**Reheating times and temperatures are usually sufficient to destroy viable cells, but are not sufficient to inactivate the heat-resistant toxins.**

**References:**


Fairly rapid cell inactivation occurs at 150°F (65.5°C), with very rapid inactivation at 165°F (73.9°C) and above. Organisms grown under stress [e.g., 115°F (46.1°C)] have higher heat resistance and survive longer in foods than those grown under optimal conditions. Table 4-22 indicates time for destruction of *S. aureus* in Chicken a la king and custard.

### Table 4-22

**Thermal Resistance of *S. aureus* 196 E in Custard and Chicken a la King**

<table>
<thead>
<tr>
<th>Temperature (°F)</th>
<th>Time Required to Inactivate 10^7 Organisms/gram (min.)</th>
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* Adapted from Angeloti et al., 1961.

Dahl et al. (1980) studied the effect of microwave heating on viability and destruction of *S. aureus* of in beef loaf and other foods. (The foods used in this study were inoculated with 10^7 microorganisms/gram of food.) They found that heating these foods to an internal temperature of 165 to 171°F (74 to 77°C) did not always eliminate viable cells of *S. aureus*. This is possible because microwave energy does not heat food uniformly. There can be a 10°F (5.5°C) to greater than 50°F (22.5°C) difference in temperature within a microwave product. Each microwave oven must be measured to determine its individual uniformity characteristics.

Multiplication of the organism is possible during cooling, when the cooling process does not proceed fast enough.

Cells of *S. aureus* survive freezing. Freezing does not reduce the potency of *S. aureus* enterotoxins.

If a food is processed with methods that eliminate *S. aureus*, then toxin will not be formed. However, the organism is so common to the human population that it must be assumed that food will probably be recontaminated with *S. aureus* after cooking. For safety, food should be maintained above 130°F (54.4°C), or be cooled continuously from 120 to 40°F (48.9 to 4.4°C) within 14 hours [Juneja et al., 1994, USDA-FSIS, 2001]. [The FDA Food Code recommends holding food at 135°F (57.2°C) or above, and cooling food from 135 to 70°F (57.2 to 21.1°C) within 2 hours, followed by cooling to <41°F (5°C) within a total cooling time of 6 hours or less.]

**Heat resistance of *S. aureus* toxins.** Toxins produced by *S. aureus* are quite heat stable. Read and Bradshaw (1966) reported a D-value for enterotoxin B, the most heat stable enterotoxin, to be 68.6 minutes at 210°F (98.9°C). This enterotoxin can withstand over an hour of boiling temperatures before it is inactivated. Thermal inactivation of enterotoxins are affected by many factors and therefore, hard-and-fast rules about destroying these toxins if present in food are not reliable. Since *Staphylococcus* toxins resist boiling temperatures for up to an hour, they cannot be destroyed by most cooking procedures. Because *Staphylococcus* toxins will not be destroyed in normal recipe operations, it can never be

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**Staphylococcus aureus – Process Critical Controls**

**Staphylococcus aureus Control**

Less than optimum conditions of pH or temperature, unfavorable atmosphere, competition from other microorganisms, or heat damage to the cells during cooking or processing all can affect the lag time, growth rate, and maximum cell population of *S. aureus*. In order to inhibit the growth of *S. aureus* and subsequent toxin production, growth conditions must be controlled.

**Temperature.** Manipulation of temperature is an effective control. Storage of food below 43°F (6°C) or above 122°F (50°C) prevents the growth of *S. aureus* and toxin production.

**Heat sensitivity of viable cells.** The viable (living) cells of *S. aureus* are more resistant than Salmonella spp. to heat and are not always inactivated during the pasteurization of milk [161°F (72.2°C) for 15 seconds] or cooking of food, especially in a microwave oven.

An increase in protein has a protective effect on staphylococcal organisms. This means that foods high in protein must be heated to a higher temperature and/or for longer periods of time in order to effect destruction.

Increasing fat has no protective action. More time is required to destroy these microorganisms in skim milk or whey than in whole milk, all heated to 140°F (60°C) (Kadan et al., 1963). Lipases produced by *S. aureus* act upon the fat to produce free fatty acids which then aid in the destruction of the microorganism.

Heat resistance varies with the strain of the organism and nature of the food. Webster and Esselen (1956) reported that *S. aureus* vegetative cells were inactivated in poultry stuffing in 0.38 minutes at 160°F (71.1°C) and in 0.15 minutes at 165°F (73.9°C). They concluded that a center temperature of 165°F (73.9°C) in poultry stuffing should be adequate to destroy any *S. aureus* present in the stuffing. The turkeys in this study were cooked in a conventional oven. To attain this temperature in the center of poultry stuffing in the interior cavity of the turkey, the white meat (breast) was cooked above its optimum temperature of 165°F (73.9°C), which undoubtedly caused it to become quite dry.

Fairly rapid cell inactivation occurs at 150°F (65.5°C), with very rapid inactivation at 165°F (73.9°C) and above. Organisms grown under stress [e.g., 115°F (46.1°C)] have higher heat resistance and survive longer in foods than those grown under optimal conditions. Table 4-22 indicates time for destruction of *S. aureus* in Chicken a la king and custard.

### Table 4-22

**Thermal Resistance of *S. aureus* 196 E in Custard and Chicken a la King**

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Cells of *S. aureus* survive freezing. Freezing does not reduce the potency of *S. aureus* enterotoxins.

If a food is processed with methods that eliminate *S. aureus*, then toxin will not be formed. However, the organism is so common to the human population that it must be assumed that food will probably be recontaminated with *S. aureus* after cooking. For safety, food should be maintained above 130°F (54.4°C), or be cooled continuously from 120 to 40°F (48.9 to 4.4°C) within 14 hours [Juneja et al., 1994, USDA-FSIS, 2001]. [The FDA Food Code recommends holding food at 135°F (57.2°C) or above, and cooling food from 135 to 70°F (57.2 to 21.1°C) within 2 hours, followed by cooling to <41°F (5°C) within a total cooling time of 6 hours or less.]

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assumed that cooking will make food safe to eat. **Heating food to 165°F will not assure destruction of toxin produced by Staphylococcus aureus.**

**Prevention of toxin formation.** Toxins are formed between 50 to 115°F (10 to 46°C). Foods must not be allowed to remain in this temperature range for long periods of time. Food must be heated from 41 to 130 (5 to 54.4°C) in less than 6 hours, and cooled to less than 45°F (7.2°C) in 15 hours or less.

The production of *S. aureus* enterotoxins must be prevented by using proper methods of sanitation, by preventing cross-contamination, and by using food preparation and storage temperatures that prevent this microorganism from multiplying and producing enterotoxin in food.

A critical step in the preparation of meat, fish, and poultry salads is to have the temperature of all ingredients below 41°F (5°C) at the start of preparation. These cold salads and other cold high protein items should be mixed sufficiently and returned to the cooling unit for storage before the temperature of these products reaches 50°F (10°C).

**pH and salt.** The minimum pH permitting growth in laboratory media is 4.5 pH in 8-10% table salt (sodium chloride) and 6.0 pH in 16% NaCl. There is no toxin production above pH 9.0 or below 5.0.

No toxin is produced, regardless of the pH, if there is 10% or more NaCl. The practical application of this knowledge is used in salted butter, where there is 12% salt in the water phase to assure its safety.

**Summary**

To prevent foodborne illnesses caused by *S. aureus* intoxication, the growth of the organism must be controlled or inhibited. Food processing and foodservice establishments should:

1. Assure that food preparers wash their hands before handling food.
2. Assure that no foodservice workers with infected cuts or boils handle food.
3. Use effective measures to prevent contamination and to control multiplication of *S. aureus* in food during its preparation, service, and storage. This includes washing and sanitizing food preparation surfaces and utensils.
4. Keep food below 41°F (5°C) and above 130°F (54.4°C). Cool cooked or partially cooked food products to less than 40°F (4.4°C) within 14 hours or less. Heat food from 41 to 130°F (5 to 54.4°C) in less than 6 hours.
5. Realize that incoming food of animal origin is often contaminated with *S. aureus* and the microorganisms are more difficult to inactivate with heat than are *Salmonella* spp. However, a cell population of 10⁶ *S. aureus* per gram of food is not a health hazard. A cell population of more than 10⁷/gram is necessary to produce sufficient toxin to cause illness.

**References:**


**CHARACTERISTICS OF CLOSTRIDIUM PERFRINGENS**

- Common in intestinal tract and feces of humans and animals, in soil and dust, and on the hides of animals at slaughter.
- Grows best without air (anaerobic) in the center of food, in large pots, in take-out food, or in lukewarm food on a buffet.
- Needs foods containing complete protein (meat, poultry, eggs) to grow; multiplies rapidly in cooked meats and gravies.
- Forms spores that are resistant to cooking / roasting temperatures; spores grow out as vegetative cells when food cools.
- Causes illness when food that is consumed contains high numbers of vegetative cells that sporulate in the intestine and release toxin.
- Heating to 145°F inactivates vegetative cells and makes food safe.

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**Clostridium perfringens** – Characteristics

**Bacterial Characteristics**

*Clostridium perfringens* is gram-positive, spore-forming, rod-shaped, encapsulated, anaerobic but aerotolerant species of bacteria.

This microorganism was first determined to cause foodborne illness in the 1940s. Previous to that time it was known to be one of the causes of gas gangrene in humans, and causes various intestinal diseases in animals. The strains of *C. perfringens* are divided according to the 5 toxin types they produce. Type A toxin strains are responsible for almost all of the foodborne illness outbreaks.

**Source**

*Clostridium perfringens* (both vegetative cells and spores) is extremely widespread in nature. It is a common inhabitant of the intestinal tract of healthy humans and animals. Spores of this bacteria are found in soil, sewage, manure, water, and dust. Therefore, many foods, especially meats, are likely to be contaminated with *C. perfringens*.

When food is cooked, the vegetative cells are destroyed and the food is safe to consume. However, if protein containing foods, such as roast beef, turkey, gravies and sauces are allowed to cool slowly and remain at temperatures which allow the germination of surviving spores, vegetative cells will multiply rapidly to large numbers in the food and create a significant hazard.

**Growth Conditions**

**Nutrients.** *Clostridium perfringens* needs a complete source of protein. It requires 13 to 14 specific amino acids to grow and is usually found in foods of animal origin such as meat and poultry. It grows rapidly in other protein-containing foods such as gravies, sauces, and soups at optimum growth temperatures.

**Temperature.** *Clostridium perfringens* is able to grow at higher temperatures than any other food pathogenic bacteria. Its growth temperature range is 59 to 127.5°F (15 to 52.3°C) (Labbe, 1989, Shoemaker and Pierson, 1976). Its optimum growth temperature is between 105 to 113°F (40.6 to 45°C). Willardson et al. (1978) reported a rapid generation time for *C. perfringens* in cooked ground beef of 7.1 minutes at 105.8°F (41°C). This is one of the most rapid generation times reported for any bacteria. Growth at these temperatures is also affected by pH, water activity, supply of nutrients, and atmosphere.

Vegetative cells (but not spores) of *C. perfringens* are significantly reduced in numbers during refrigeration and frozen storage (Canada et al., 1964). Trakulchang and Kraft (1977) found that 98.5% of the vegetative cells in different foods were inactivated after 28 days at 0°F (-18°C) while the spore count did not change.

**Atmosphere.** *Clostridium perfringens* is an anaerobic bacterium but can adapt to grow in air (oxygen environment), particularly during the log phase (Labbe, 1989). This means that *C. perfringens* can grow and survive on the surface of foods as well as in the center of products, such as a large kettle of gravy or soup.

**Water activity.** The lowest water activity supporting growth of *C. perfringens* is between 0.97 to 0.95 a_w. The lowest water activity for sporulation is 0.98 a_w (Labbe, 1989).

**pH.** *Clostridium perfringens* grows between pH 5.0-9.0 (Fuchs and Bonde, 1957). Its optimum pH for growth is between 6.0 and 7.0 (Labbe, 1989). As cells grow in foods, the pH decreases, and after a period of a few days cells may die off as a result.

**Other factors.** Nitrites can be used to inhibit or prevent the growth of *C. perfringens*. The amount of nitrite necessary is dependent on the pH of the system, amount of salt and heat processing after addition of nitrite. Cured meat products are rarely involved in *C. perfringens* foodborne illness.

Levels of 5 to 8% salt are sufficient to prevent the growth of most strains of *C. perfringens* (Craven, 1980).

**Factors Affecting Sporulation and Toxin Formation**

Sporulation is the formation of spores by bacterial cells. Enterotoxin type A is formed and foodborne illness (gastroenteritis) develops when a large number of viable cells of *C. perfringens* sporulate in food, or are eaten, pass through the stomach and sporulate in the intestine.

**Effect of Heat on Clostridium Perfringens**

While temperatures of 140°F (60°C) are quite effective in inactivating vegetative cells, a temperature of 180°F (82.2°C) during cooking stimulates (heat shocks) *C. perfringens* spores into activation. The spores then germinate to form viable cells when food products cool below 130°F (54.4°C).

Spores in food products are more heat stable than vegetative cells. The heat resistance of spores of *C. perfringens* varies between strains. Bradshaw et al. (1977) found that the D value (decimal destruction time) for *C. perfringens* type A spores was 27 to 30 minutes in beef gravy at 210°F (99°C).

The D value for vegetative cells of *C. perfringens* grown at higher temperatures 113 to 120°F (45 to 49°C) is 7.2 minutes at 138°F (58.9°C) (Roy et al., 1981).

**Cause of Illness**

When food containing large numbers of vegetative cells of *C. perfringens* is consumed, the cells pass through the stomach to the intestine where they sporulate and release an enterotoxin. The enterotoxin binds to the cell membranes in the intestine...
causing rapid water and ion changes in the intestinal mucosa resulting in diarrhea.

Naik and Duncan (1977) reported that sporulation and enterotoxin production can occur in foods. They were also able to detect enterotoxin in a food product involved in a foodborne illness incident. Early development of symptoms (1 to 2 hours) involved in some outbreaks of C. perfringens can be explained by the presence of preformed enterotoxin in food that is formed when vegetative cells sporulate in food.

As spores and vegetative cells of C. perfringens are shed in feces from the humans or animals, the microorganism can spread to the surrounding environment and become a source of illness once more.

**Symptoms**
Illness usually occurs 8 to 24 hours after ingestion of food containing appropriate numbers of vegetative cells. Symptoms include diarrhea and severe abdominal pain. Symptoms of nausea, vomiting, or fever are unusual. The illness lasts 12 to 24 hours and fatalities are extremely rare, occurring only in chronically ill, institutionalized, or elderly individuals.

**Infective Dose**
To cause illness, vegetative cells must multiply to large numbers in the food. Chin (2000) lists the infective dose of C. perfringens as greater than 10⁶ microorganisms per gram of food. The FDA (1993) lists the infective dose as greater than 10⁹ microorganisms.

**Incidence**
Mead et al., (1999) estimates there are around 250,000 cases of Clostridium perfringens enteritis that occur annually in the United States. The illness is rarely fatal.

**Outbreaks**
Foodborne disease outbreaks frequently have followed ingestion of turkey served in institutional and commercial foodservice establishments and in homes. In a typical episode, 80% of students and teachers who ate in a school cafeteria experienced a mild gastroenteritis characterized by abdominal cramps and diarrhea. Clostridium perfringens was identified from 13 to 14 isolates cultured from stool specimens from 5 patients. Food histories indicated that the vehicle was either turkey or dressing.

Two days before the outbreak, frozen turkeys had been thawed at room temperature in a school kitchen. The following day some were cooked in a steamer and others in large pots on a range. The cooked turkeys and the liquid in which they were cooked (stock) were cooled on tabletops for about an hour before the bones were removed and the stock transferred to 1-gallon jars. The stock and meat were then stored in a refrigerator overnight. During this period, the stock (in gallon containers) and boned meat (in 6-inch pans) did not cool immediately, and thus were within a temperature range suitable for multiplication of C. perfringens for over 8 hours.

The next morning, the stock was heated and some mixed with cornbread crumbs and other ingredients to make dressing that was baked to a temperature of 212°F (100°C). The cold turkey was diced, and gravy that had been made from the remainder of the stock was poured over it. Although this mixture was warmed in ovens, it did not reach a hot enough temperature to destroy the large numbers of vegetative cells of C. perfringens that were present, and therefore, was the vehicle in this outbreak.

**OUTBREAK EXAMPLE.** The following example appeared in MMWR 43 (08): 137-138, 143-144.

**Clostridium perfringens** gastroenteritis associated with corned beef served at St. Patrick's Day Meals -- Ohio and Virginia, 1993.

**Ohio.** On March 18, 1993, the Cleveland City Health Department received telephone calls from 15 persons who became ill after eating corned beef purchased from one delicatessen. After a local newspaper article publicized this problem, 156 persons contacted the health department to report onset of diarrheal illness within 48 hours of eating food from the delicatessen on March 16 or March 17. Symptoms included abdominal cramps (88%) and vomiting (13%); no persons were hospitalized. The median incubation period was 12 hours (range: 2 to 48 hours). Of the 156 persons reporting illness, 144 (92%) reported having eaten corned beef; 20 (13%), pickles; 12 (8%), potato salad; and 11 (7%) roast beef.

In anticipation of a large demand for corned beef on St. Patrick's Day (March 17), the delicatessen had purchased 1400 pounds of raw, salt-cured product. Beginning March 12, portions of the corned beef were boiled for 3 hours at the delicatessen, allowed to cool at room temperature, and refrigerated. On March 16 and 17, the portions were removed from the refrigerator, held in a warmer at 120°F (48.8°C), and sliced and served. Corned beef sandwiches were also made for catering to several groups on March 17; these sandwiches were held at room temperature from 11 a.m. until they were eaten throughout the afternoon.

**Virginia.** On March 28, 1993, 115 persons attended a traditional St. Patrick's Day dinner of corned beef and cabbage, potatoes, vegetables, and ice cream. Following the dinner, 86 (76%) of 113 persons interviewed reported onset of illness characterized by diarrhea (98%), abdominal cramps (71%), and vomiting (5%). The median incubation period was 9.5 hours (range: 2 to 18.5 hours). Duration of illness ranged from 1 hour to 4.5 days. One person was hospitalized.

Corned beef was the only food item associated with illness. Cultures of stool specimens from 8 symptomatic all yielded 10⁶ or more colonies of C. perfringens per gram. A refrigerated sample of leftover corned beef yielded 105 or more colonies of C. perfringens per gram.

The corned beef was a frozen, commercially brined product. Thirteen pieces, weighing approximately 10 pounds each, had been cooked in an oven in four batches during March 27-28. Cooked meat from the first three batches was stored in a home refrigerator. The last batch was taken directly to the event. Approximately 90 minutes before serving began the meat was sliced and placed under heat lamps.

The errors in preparation of the corned beef in these outbreaks were typical of those associated with previously reported foodborne outbreaks of C. perfringens. Improper holding temperatures were a contributing factor in most (97%) outbreaks reported to the CDC from 1973 to 1997. To avoid
illness caused by this organism, food should be eaten while still hot or reheated to an internal temperature $\geq 165^\circ F$ ($\geq 74^\circ C$) before serving.

References:
FDA, 1993. HACCP. Regulatory Food Applications in Retail Food Establishments. Dept. of Health and Human Services. Division of Human Resource Development, HFC-60; Rockville, MD.
**Clostridium perfringens – Process Hazard Analysis**

**Typical Hazards**  
Hazardous foods include improperly cooled beef, turkey, Mexican food (tacos, enchilada, and beans), casserole items, gravies, and soups containing adequate sources of protein.  

*Clostridium perfringens* is transferred to meat and poultry from animal feces during processing, from the hands of workers that have not been washed after using the toilet, from soil and dust, and from unsanitized food preparation equipment and surfaces. Other food products such as vegetables and spices are often contaminated with *C. perfringens* (both spores and vegetative cells), and when added to meat and poultry products can lead to direct contamination of these products.  

Although the presence of small numbers of *C. perfringens* in many foods is unavoidable, large numbers may be indicative of mishandling and temperature abuse.  

In the Cincinnati area, *C. perfringens* was found in raw and some partially cooked meat and meat products, and some fully processed meat products requiring no cooking. The organism was isolated from 43.1% of 262 specimens. The highest percentage of contamination was found in veal cuts, and the lowest in sandwich cuts and spreads. Only 2 of the 113 isolates produced heat-resistant spores (Hall and Angelotti, 1965).  

An analysis of dehydrated soups and sauces for *C. perfringens* indicated that 18.2% of the samples were contaminated (Nakamura and Kelly, 1968). The presence of *C. perfringens* in low numbers (less than 10^2/gram) is common. Large numbers (more than 10^5/gram) in a food are cause for concern.  

**Temperature Abuse**  
Food will always have spores that survive cooking and will form vegetative cells that multiply during improper hot holding and slow cooling. Prime rib of beef held on cutting boards at temperatures below 130°F (54.4°C) will rapidly become hazardous. For example, if 1,000 cells of *C. perfringens* per gram in a food product containing a high quality protein source remain at 109 to 113°F (43 to 45°C) for 2 hours 30 minutes, the cells can multiply to a hazardous level of 10^6 per gram of food.  

**Detection**  
An adequate and official AOAC method is available for detection and enumeration of *C. perfringens* in foods. However, several factors limit its application in routine surveillance of foods. If food samples are held in freezers or under refrigeration, vegetative cells of *C. perfringens* lose viability (die off).  

Foods that are suspect vehicles in gastroenteritis outbreaks and in which *C. perfringens* is likely to be found should be examined quantitatively. A detectable quantity of alpha toxin is produced when cell numbers reach greater than 10^5 *C. perfringens* cells per gram. Alpha toxin is little affected by freezing or refrigerated storage of foods. There are test kits available for analyzing the presence of this toxin.

**References:**  
FDA, 1993. HACCP. Regulatory Food Applications in Retail Food Establishments. Dept. of Health and Human Services. Division of Human Resource Development, HFC-60; Rockville, MD.  
Clostridium perfringens – Process Critical Controls

**Clostridium perfringens Control**

_Clostridium perfringens_ foodborne illness outbreaks result when this microorganism is allowed to multiply to large numbers (more than $10^5$) during slow cooking, inadequate cooling and improper hot holding (i.e., the failure to keep hazardous foods contaminated with spores and viable cells out of critical growth range temperatures of 59 to 127.5°F (15 to 52.3°C) for extended periods of time).

**Control Factors**

Food production and foodservice establishments must:

1. Use cooking methods that ensure that food products, particularly large roasts, poultry items, and high protein casseroles, pass a center temperature between 80 to 130°F (26.7 to 54.4°C) in 2 hours or less [40 to 130°F (4.4 to 54.4°C) in 6 hours or less].

2. For safety, food should be maintained above 130°F (54.4°C), or be cooled continuously from 120 to 40°F (48.9 to 4.4°C) within 14 hours [Juneja et al., 1994, USDA-FSIS, 2001]. [The FDA Food Code recommends holding food at 135°F (57.2°C) or above, and cooling food from 135 to 70°F (57.2 to 21.1°C) within 2 hours, followed by cooling to <41°F (5°C) within a total cooling time of 6 hours or less.]

3. When cooling products:
   a. Large roasts should be sliced and place in layers not thicker than 2 inches in depth.
   b. Stews, soups, casserole items, gravies, and sauces should be placed in pans that are not thicker than 2 inches in depth for cooling.
   c. High velocity fans should be used in the cooling or refrigeration systems to insure rapid air circulation.

4. Hold hot protein items above 130°F (54.4°C). (FDA regulations for holding food above 140°F (60°C) provide an extra measure of safety because of the unreliability of thermostats on hot holding equipment).

5. Train foodservice personnel to practice 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.

6. Use sanitized equipment to penetrate 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. Penetrating objects include cook's forks, thermometers, and metal spits used to rotate the meat.

References:


FDA, 1993. HACCP. Regulatory Food Applications in Retail Food Establishments. Dept. of Health and Human Services. Division of Human Resource Development, HFC-60; Rockville, MD.


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 (29.4 to 48.9°C) for a long period of time (e.g. the number of hours typical for 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.

Source

Bacillus cereus is commonly in 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 foodstuffs. For example, Bacillus 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 Goepfert, 1971).

Growth Conditions

Temperature. Psychrotrophic strains of this pathogen have been shown to grow at a temperature range of 39.2 to 98.6°F (4 to 37°C) in pasteurized milk, mousses, and cook/chill meals (van Netten et al., 1990).

The maximum temperature for vegetative cell growth and spore outgrowth is 122°F (50°C) (Kramer and Gilbert, 1989). Optimum growth occurs at temperatures of 82 to 95°F (28 to 35°C) (Adams and Moss, 1995). Wong et al. (1988) reported a generation time of 27 minutes in pasteurized milk at 86°F (30°C). Johnson et al. (1983), reported a generation time of 26 to 57 minutes at 86°F (30°C) 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 (cooked) often contains 100 vegetative cells per gram as well as spores of B. cereus. This population is capable of multiplying to 10^5 microorganisms in as little as 300 minutes (5 hours) at 86°F (30°C).

Nutrients. Bacillus cereus produces enzymes than can hydrolyze (split) starch and protein. It thus uses 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 (100°C) 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 10^3 to 10^6 spores, 1 may survive after 4 hours at 275°F (135°C) (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 that 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 (56°C) = 5 minutes.)* The enterotoxin associated with the emetic illness is quite heat resistant and is stable to 250°F (121.2°C) 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 x 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**

*Bacillus cereus* was first reported as a cause of foodborne illness in Norway in 1950.

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 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**

Mead et al *(1999)* estimate that *B. cereus* is a cause of over 27,000 cases of foodborne illness in the U.S. each year.

**Outbreaks**

The first well-documented incident in the U.S. occurred in 1969. Meat loaf contaminated with 7 x 10^6 *B. cereus*/gram was the vehicle for transmission of the diarrheal illness involving 15 people.

**OUTBREAK EXAMPLE.** The following example appeared in MMWR 43 (10):177-178; 1994.

*Bacillus cereus* food poisoning associated with fried rice at two child day care centers -- Virginia, 1993. On July 21, 1993, the Lord Fairfax (Virginia) Health District received reports of acute gastrointestinal illness that occurred among children and staff at two jointly owned child day care centers following a catered lunch.

The catered lunch was served on July 21 to 82 children aged years or less, and to 9 staff members. Dietary histories were obtained for 80 persons (staff and parents were question for children less than 4 years. Of the 80 persons, 67 ate the catered lunch. A case was defined as vomiting by a person who was present at either day care center on July 21. Fourteen (21%) persons who ate the lunch became ill compared with none of 16 who did not. Symptoms included nausea (71%), abdominal cramps or pain (36%), and diarrhea (14%). Twelve of the 14 cases occurred among children aged 2.5 to 5 years and two cases occurred among staff. The median incubation period was 2 hours (range 1.5-3.5 hours). Symptoms resolved a median of 4 hours after onset (range: 1.5 to 22 hours).

Chicken fried rice prepared at a local restaurant was the only food significantly associated with illness. Illness occurred in 14 (29%) of 48 persons who ate chicken fried rice, compared with none of 16 who did not. *Bacillus cereus* was isolated from leftover chicken fried rice (more than 10^6 organisms / gram) and from the vomitus of one child.

The rice had been cooked the night of July 20 and cooled at room temperature before refrigeration. On the morning of the lunch, the rice was pan-fried in oil with pieces of cooked chicken, delivered to the day care centers at approximately 10:30 a.m., held without refrigeration, and served at noon without reheating.

Fried rice has been implicated in many outbreaks of *B. cereus* emetic-type food poisoning in the United States. *B. cereus* is frequently present in uncooked rice, and heat-resistant spores may survive cooking. If cooked rice is subsequently held at room temperature, vegetative forms multiply, and heat-stable toxin is produced that can survive brief heating, such as stir frying. In the outbreak described in this report, vegetative forms of the organism probably multiplied at the restaurant and the day care centers while the rice was held at room temperature.

Following the outbreak, health officials of Lord Fairfax Health district recommended to day care staff and restaurant food handlers that the practice of cooling rice or any food at room temperature be discontinued, food be maintained at proper temperatures [e.g., below 41°F (5°C) or above 140°F (60°C)], and a thermometer be used to verify temperature.

**Other Incidents**

The emetic syndrome has almost always been associated with fried rice served in Oriental restaurants. However, a well-documented outbreak of the emetic syndrome of *B. cereus* in a British prison implicated beef stew. This was thought to have been caused by adding vegetables that had been cooked the previous day to the stew.

Under-reporting of outbreaks of *B. cereus* is likely because illness associated with *B. cereus* is usually self-limiting and not severe.
References:
FDA, 1993. HACCP. Regulatory Food Applications in Retail Food Establishments. Dept. of Health and Human Services. Division of Human Resource Development, HFC-60; Rockville, MD.
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. 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.
3. 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.
4. Check temperatures in hot holding devices and in cooling units to assure that this equipment functions properly and repair or replace it as needed.
5. 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 continuously from 120 to 40°F (48.9 to 4.4°C) within 14 hours [Juneja et al., 1994, USDA-FSIS, 2001]. [The FDA Food Code recommends cooling food from 135°F to 70°F within 2 hours, followed by cooling to <41°F (5°C) within a total cooling time of 6 hours or less.]

The FDA recommends holding hot food at 135°F (57.2°C) or above, and cold foods at or below 41°F (5°C). Cooked food products should not be stored at room temperature.
6. Reheat leftovers and/or partially prepared items to 165°F (73.9°C) or above. Reheating food to 165°F (73.9°C) or above eliminates vegetative cells, however, it does not inactivate the spores of Bacillus cereus nor destroy emetic toxin if it has formed.
7. Use methods that adequately clean and sanitize surfaces, equipment, and utensils.

References:


**Clostridium botulinum** – Characteristics

**Bacterial Characteristics**

Clostridium botulinum microorganisms are anaerobic, spore-forming, gram-positive rods that are motile by means of flagella. There are 7 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 4 categories: (Pierson and Reddy, 1988; FDA, 1993)

1. Classical foodborne botulism intoxication caused by the ingestion of small amount of preformed botulinal 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 which 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 *Clostridium 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 or 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 *C. 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 (10 to 48°C) (Hauschild, 1989). Ohye and Scott (1953) found the optimal temperature range for growth to be 98.6 to 104°F (37 to 40°C). At 98.6°F (37°C), the generation time is 0.7 hours (42 minutes). Table 4-23 indicates the generation times for these strains at various temperatures.

Gibson et al. (1987) reported a generation time for *Clostridium botulinum* type A in pasteurized pork slurry of 1.2 hours at 68°F (20°C).

**Table 4-23**

<table>
<thead>
<tr>
<th>Temperature °F (°C)</th>
<th>Approx. lag time (hr)</th>
<th>Approx. generation time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>54 (12.2)</td>
<td>**</td>
<td>89</td>
</tr>
<tr>
<td>59 (15)</td>
<td>160</td>
<td>28</td>
</tr>
<tr>
<td>64 (17.8)</td>
<td>63</td>
<td>8</td>
</tr>
<tr>
<td>8 (20)</td>
<td>32</td>
<td>4</td>
</tr>
<tr>
<td>77 (25)</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>86 (30)</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>98.6 (37)</td>
<td>5</td>
<td>0.7</td>
</tr>
<tr>
<td>108.5 (42.5)</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>113 (45)</td>
<td>**</td>
<td>2</td>
</tr>
</tbody>
</table>

* Adapted from data of Ohre 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.

The temperature range for growth of type E and non-proteolytic type B strains is 38 to 113°F (3.3 to 45°C). 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. (See Table 4-23.)

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 *C. 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 pork slurry at a pH of 4.30 to 4.36. He postulated that *C. botulinum* was able to grow and produce toxin at a higher pH within precipitated protein matrices.

Types E and non-proteolytic 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 non-proteolytic strains of type B, *C. botulinum*, and 0.97 for type E and non-proteolytic 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 ascorbic acid.

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.

Table 4-24 summarizes some growth conditions for *C. botulinum*.

**Heat Resistance 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 (121.1°C) [D250]. Spores of non-proteolytic types E, B, and F are less heat resistant and are destroyed at 180°F (82.2°C) [D180] in 0.8 to 6.6 minutes in various foods (Simunovic et al., 1985).

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 botulinal toxin was inactivated if it was heated to 174°F (78.9°C) for 20 minutes [D254 = 20 minutes] or 185°F (85°C) 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 botulinal toxins are the most toxic substances known. A very small amount (a few nanograms) in food can cause illness. Botulinal 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 per 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.

From 1973 through 1996 in the United States, 724 cases of foodborne botulism [median, 24 cases annually (range, 8 to 86 cases)], 103 cases of wound botulism [median, 3 cases annually (range, 0 to 25 cases)], 1,444 cases of infant botulism [median, 71 cases annually (range, 0 to 99 cases)], and 39 cases of botulism of undetermined type were reported to the CDC (Shapiro et al., 1998).

Mead et al. (1999) estimate that there are about 58 cases of foodborne botulism resulting in 4 deaths, each year.

**Food Analysis**
Foodborne botulism 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 EXAMPLE**. The following outbreak example appeared in MMWR 44 (11): 200-201, 1995.

**Foodborne Botulism -- Oklahoma, 1994.** On June 30, 1994, a 47-year-old resident of Oklahoma was admitted to an Arkansas hospital with subacute onset of progressive dizziness, blurred vision, slurred speech, difficulty swallowing, and nausea. Examination showed ptosis, extraocular palsies, facial paralysis, palatal weakness, and impaired gag reflex. Analysis of a stool sample obtained on July 5 detected type A toxin, and culture of the stool yielded *C. botulinum*. The patient was hospitalized 49 days, including 42 days on mechanical ventilation, before being discharged.

The patient had reported, that during the 24 hours before onset of symptoms he had eaten home-canned green beans and a stew containing roast beef and potatoes. Although analysis of the leftover green beans was negative for botulism toxin, type A toxin was detected in the stew. The stew had been cooked, covered with a heavy lid, and left on the stove for 3 days before being eaten without reheating. No other persons had eaten the stew.

**Other outbreak 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 botulinal 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 cased 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 un gutted, salted whitefish occurred in the fall 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, un gutted, 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 which 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.

References:
FDA, 1993. HACCP. Regulatory Food Applications in Retail Food Establishments. Dept. of Health and Human Services. Division of Human Resource Development, HFC-60; Rockville, MD.
**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 *Clostridium botulinum* spores.

In general, the presence of *C. botulinum* and/or its toxins in canned foods indicated 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. Botulinic 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 insure 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 in 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 which do not exceed 212°F (100°C). 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 (2.8°C) or below.
4. Fresh vegetables (e.g., mushrooms) must be packaged in containers or bags which allow air (oxygen) to enter the packages when they are stored at temperatures of 50°F (10°C) or above.

**References**


Heating food to 165°F for 15 seconds will inactivate 10^6 to 10^7 pathogens in poultry cooked to temperatures above 165°F (73.9°C). Fortunately, most people in the United States prefer meat, fish, and poultry cooked to temperatures above 165°F (73.9°C). Heating food to 165°F for 15 seconds will inactivate 10^6 to 10^7 pathogens in poultry cooked to temperatures above 165°F (73.9°C).

Pathogen HACCP Summary – Infective, Spore-forming, and Exotoxic Foodborne Pathogens

Pathogenic Microorganism Control – Summary

From the standpoint of foodservice system HACCP analysis, it is really not necessary to know each microorganism because the microbiological hazards and control points can be grouped into 3 major categories:

1. Infective microorganism control
2. Spore-forming microorganism control
3. Exotoxic vegetative cell control.

When these three categories of microbiological hazards are controlled and numbers of pathogens in foods are kept below threshold illness levels for customers, the foods will be safe to eat.

Infective Microorganisms (Salmonella spp., Viruses, Parasites, etc.)

Because of the lack of adequate control in the raw food wholesale system, it must be assumed that the meat, fish, poultry, vegetables, fruits, starches, spices, etc., at receiving, are sufficiently contaminated to make some consumers ill. For immune-compromised persons, 1 microorganism per 25 grams of food can make them ill. For healthy people, it may take 100,000 microorganisms to make them ill. The critical control procedures are:

1. Cooking food adequately in order to reduce microbial populations to a safe level.
2. Preventing cross-contamination and multiplication of microorganisms in foods after cooking.

If the food (e.g., beef, pork, fish) is to be served raw or not cooked sufficiently to pasteurize the product [heating to 140°F (60°C) for 8.65 minutes], it must be purchased from growers or suppliers who certify the microbiological safety of their products. When food is received randomly from unknown growers or harvesters through the wholesale system, it must be assumed that the food has levels of pathogens sufficient to cause illness. The food must be pasteurized.

Fortunately, most people in the United States prefer meat, fish, and poultry cooked to temperatures above 165°F (73.9°C). Heating food to 165°F for 15 seconds will inactivate 10^6 to 10^7 infective microorganisms per gram of food and make the food safe to eat.

Demuth (1990) studied the pathogenic microorganisms that occur in ready-to-eat foods and found that salmonellae, shigellae, and staphylococci are the most common. Salmonellae and shigellae cause diarrheal diseases, and staphylococci cause gastrointestinal illness. These pathogens are found in meat, poultry, eggs, and dairy products.

Populations of infective microorganisms on fruits and vegetables are decreased by washing these foods in flowing water. Dilute or mild (50 to 400 ppm) chlorine (bleach) solutions should not be used in water used to wash fruits and vegetables because a residual amount of chlorine may remain on the food. At this time, there is concern about the toxicity of chlorine ingestion.

Another critical problem is the recontamination of cooked food with infective organisms from inadequately washed hands and unwashed, unsanitized cutting boards and knives, followed by sufficient time and temperature for the organisms to multiply to hazardous levels. Separate cutting boards and knives, and proper surface and hand sanitizing procedures can control this problem.

Infective organisms on raw food. Freezing at below 0°F (-17.8°C) for 5 days is an effective method for inactivating parasites such as *Trichinella spiralis*, but is not an effective method for inactivating bacteria and viruses.

Populations of infective microorganisms in food. Even the best microbial standards permit 1 microorganism per 500 grams of food. Infective microorganisms must be controlled with heat, freezing, washing, knowledge of the grower or harvester, etc., to reduce the population level to below the threshold that makes consumers ill.

Spore-forming, Exotoxin-Producing Microorganisms (*Clostridium perfringens*, *Clostridium botulinum*, *Bacillus cereus*), and Exotoxin-Producing *Staphylococcus aureus*

All food has spores of one type or another. Meat may contain spores of *Clostridium perfringens*, *Clostridium botulinum* and *Bacillus cereus*. Spores of *C. botulinum* are commonly found in vegetables; *B. cereus* is often present in cereals, grains and milk. While the vegetative cells of these organisms are all more sensitive to heat than the infective vegetative cells, the spore forms of these organisms are very resistant to heat processes encountered during most cooking procedures. The spores actually become activated during cooking. When cooked products are not held at temperatures above 130°F (54.4°C) or are cooled slowly, the spores germinate to form...
vegetative cells which can then multiply to hazardous levels. A vegetative cell population of greater then $10^5$ cells per gram of food is usually required to cause an illness. This vegetative cell population increase occurs over a period of time. The growth of *C. perfringens* stops below a temperature of 59°F (15°C). The vegetative cell growth of *C. botulinum* type A and proteolytic type B strains is halted at temperatures below 50°F (10°C).

However, type E and non-proteolytic type B strains of *Clostridium botulinum* can grow as low as 38°F (3.3°C). Vegetative cell growth of *Bacillus cereus* is not stopped until a temperature below 39°F (3.9°C) is reached. Because of spore survival during cooking and ability to grow at low temperatures, food containing these pathogens can be hazardous and cause illness. Foods must be cooled rapidly and should be stored at temperatures near 30°F (-1.1°C).

When big pots of meat stock, which take days to cool and probably have millions of *Clostridium perfringens* per gram, are brought to a boil before using or serving, the vegetative cells are inactivated. This amount of cooking ensures the safety of the stock from cells of *Clostridium perfringens*. However, there is no way to be sure that other toxin-producing microorganisms did not grow.

HITM [based on research by Juneja et al., 1994] recommends that when food, not more than 2 inches in depth is cooled, continuously, in a standard refrigerator, from 120 to 40°F (48.9 to 4.4°C) within 14 hours it will be safe. [The FDA Food Code recommends cooling food from 135 to 70°F (57.2 to 21.1°C) within 2 hours, followed by cooling to <41°F (5°C) within a total cooling time of 6 hours or less.]

**Exotoxic Vegetative Cell Microorganisms**

*(Staphylococcus aureus, Bacillus cereus)*

These toxin-producing organisms must multiply to high numbers and produce toxins in food to make people ill. This takes time, probably more than 8 hours, at a reasonable temperature of 80 to 115°F (26.6 to 46.1°C).

A vegetative cell population of 100 to 1,000 *Staphylococcus aureus* cells per gram is usually found in food. When foods are allowed to remain 80 to 115°F (26.6 to 46.1°C), only 10 to 12 generations are required before the food becomes hazardous. Since *S. aureus* can have a generation time of 15 minutes in milk and egg products such as custards, foods must be kept hot or cooled rapidly and kept cold to prevent *S. aureus* growth and toxin production.

There is the almost certain contamination of food with a few *S. aureus* every time an employee handles a food item. *S. aureus* is not a problem on the raw food coming into the kitchen, because growth is inhibited by spoilage microorganisms. Cooking destroys spoilage microorganisms. Therefore, when cooked foods are handled and contaminated with *S. aureus*, there is no competitive inhibition; growth and toxin production of *S. aureus* can occur when conditions are favorable. If cooled food is held below 50°F (10°C) or above 115°F (46.1°C), toxin production of *Staphylococcus aureus* will be controlled.

The FDA Food Code lists reheating of cooked food to 165°F (74°C) for 15 seconds as a critical control procedure. Control of time and temperature during food handling to prevent microorganism growth is the only strategy to use to assure safety.

References


Summary of Critical Temperatures for Control

Critical Temperatures

The range for critical temperatures in foodservice is from 30 to 127.5°F. Multiplication of some spoilage microorganisms occurs above and below this range, but no pathogenic microorganisms will multiply outside of this range.

23°F Spoilage bacteria begin to multiply. Enzymatic activity causes deterioration of frozen food, even down to -40°F.

28.5°F Meat, fish, and poultry begin to thaw.

30°F *Yersinia* spp., *Listeria monocytogenes*, and *Aeromonas hydrophila* begin to multiply (29.3°F). *Escherichia coli* and *Clostridium botulinum* (type E) begin to multiply. *Bacillus cereus* begins to multiply at 39.2°F.

41°F Food prepared in a foodservice facility should not be held longer than 10 days in order to assure safety from the pathogens, especially *Listeria monocytogenes*, that multiply below this temperature. Some *Salmonella* spp. also begin to multiply at this temperature.

43°F *Staphylococcus aureus* begins to multiply, but it does not produce a toxin until the temperature of the food goes above 50°F. The temperature range of 40 to 50°F allows food to be out of the refrigerator for a short period of time during preparation in a kitchen. Thirty minutes is probably a reasonable time limit for preparing food before it is cooked or returned to the refrigerator. Food should always be returned to the refrigerator at less than 50°F unless it is cooked immediately.

50°F *S. aureus* begins to produce toxin. *Clostridium botulinum* (types A and B) begin to multiply.

59°F *Clostridium perfringens* begins to multiply.

95-97°F The temperature of rapid multiplication for most pathogenic bacteria.

105.8°F *Clostridium perfringens* can multiply once every 7.1 minutes in ground beef.

115°F Most vegetative cells stop multiplying.

122°F *Staphylococcus aureus* and *B. cereus* stop growing.

127.5°F *Clostridium perfringens* stops multiplying. This is the highest growth temperature for a pathogen.

130°F Vegetative infective pathogens such as *Salmonella* spp. can be reduced from 3,160,000 microorganisms per gram of food is reduced to less than 1 per gram (6.5D) in 112 minutes. This is the lowest temperature and time to which food should ever be cooked.

140°F Destruction of *Salmonella* spp. is 10 times faster than at 130°F. At 140°F, 3,160,000 microorganisms per gram of food is reduced to less than 1 per gram (6.5D) in 11.2 minutes.

150°F At this temperature, a population of 3,160,000 *Salmonella* spp. per gram of food is reduced to 1 per gram (6.5D) in 1.12 minutes (67 seconds).

160°F Rapid destruction of pathogenic vegetative infective microorganisms such as *Salmonella* spp. occurs. 3,160,000 *Salmonella* spp. per gram of food are reduced to 1 per gram (6.5D) in 0.112 minute (6.7 seconds). Some spoilage microorganisms survive heat at this temperature. These vegetative cells remain in the food and cause it to spoil during refrigerator storage.

212°F All vegetative cells are destroyed but spores survive. Toxins that may have been formed during the growth phase of *S. aureus* and *B. cereus* will remain unchanged and toxic for hours at this temperature.

250°F This is the temperature for sterilization of food. Spores of *C. botulinum* at a concentration of 10^{12} per ml in the center of a can of food are reduced to 1 in 3 minutes during the commercial canning of food.