Bad Bug Book

Handbook of Foodborne Pathogenic Microorganisms and Natural Toxins

Introduction

Food safety is a complex issue that has an impact on all segments of society, from the general public to government, industry, and academia. The second edition of the Bad Bug Book, published by the Center for Food Safety and Applied Nutrition, of the Food and Drug Administration (FDA), U.S. Department of Health and Human Services, provides current information about the major known agents that cause foodborne illness. The information provided in this handbook is abbreviated and general in nature, and is intended for practical use. It is not intended to be a comprehensive scientific or clinical reference.

Under the laws administered by FDA, a food is adulterated if it contains (1) a poisonous or otherwise harmful substance that is not an inherent natural constituent of the food itself, in an amount that poses *a reasonable possibility* of injury to health, or (2) a substance that is an inherent natural constituent of the food itself; is not the result of environmental, agricultural, industrial, or other contamination; and is present in an amount that *ordinarily* renders the food injurious to health. The first includes, for example, a toxin produced by a fungus that has contaminated a food, or a pathogenic bacterium or virus, if the amount present in the food *may be* injurious to health. An example of the second is the tetrodotoxin that occurs naturally in some organs of some types of pufferfish and that *ordinarily* will make the fish injurious to health. In either case, foods adulterated with these agents are prohibited from being introduced, or offered for introduction, into interstate commerce.

Our scientific understanding of pathogenic microorganisms and their toxins is continually advancing. When scientific evidence shows that a particular microorganism or its toxins can cause foodborne illness, the FDA may consider that microorganism to be capable of causing a food to be adulterated. Our knowledge may advance so rapidly that, in some cases, an organism found to be capable of adulterating food might not yet be listed in this handbook. In those situations, the FDA still can take regulatory action against the adulterated food.

The agents described in this book range from live pathogenic organisms, such as bacteria, protozoa, worms, and fungi, to non-living entities, such as viruses, prions, and natural toxins. Included in the chapters are descriptions of the agents' characteristics, habitats and food sources, infective doses, and general disease symptoms and complications. Also included are examples of outbreaks, if applicable; the frequency with which the agent causes illness in the U.S.; and susceptible populations. In addition, the chapters contain brief overviews of the analytical methods used to detect, isolate, and/or identify the pathogens or toxins.

However, while some general survival and inactivation characteristics are included, it is beyond the scope of this book to provide data, such as D and z values, that are used to establish

processes for the elimination of pathogenic bacteria and fungi in foods. One reason is that inactivation parameters for a given organism may vary somewhat, depending on a number of factors at the time of measurement. For more information on this topic, readers may wish to consult other resources. One example is the International Commission on Microbiological Specifications for Foods, the source of a comprehensive book (*Microorganisms in Foods 5. Characteristics of Microbial Pathogens*) on the heat resistance (D and z values) of foodborne pathogens in various food matrices, as well as data on survival and growth in many foods, including data on water activity and pH.

The Bad Bug Book chapters about pathogenic bacteria are divided into two main groups, based on the structure of the microbes' cell wall: Gram negative and Gram positive. A few new chapters have been added, reflecting increased interest in certain microorganisms as foodborne pathogens or as potential sources of toxins.

Another new feature is the brief section for consumers that appears in each chapter and is set apart from the main text. These sections provide highlights of information, about the microbe or toxin, that will be of interest to consumers, as well as information and links regarding safe food-handling practices. A glossary for consumers is included at the end of the book, separately from the technical glossary.

Various chapters link readers to Federal agencies with an interest in food safety, including the FDA, the Centers for Disease Control and Prevention (CDC), and the U.S. Department of Agriculture Food Safety Inspection Service. These are the primary agencies that collaborate to investigate outbreaks of foodborne illness, prevent foodborne illness, and advance the field of food safety, to protect the public's health. In addition, some technical terms have been linked to the National Library of Medicine's Entrez glossary.

Links to recent articles from the CDC's Morbidity and Mortality Weekly Reports are provided in selected chapters, to provide readers with current information about outbreaks or incidents of foodborne disease. At the end of selected chapters about pathogenic microorganisms, hypertext links are included to relevant Entrez abstracts and GenBank genetic loci.

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Introduction for Consumers: A Snapshot

Each chapter in this book is about a pathogen – a bacterium, virus, or parasite – or natural toxin that can contaminate food and cause illness. The book was prepared by the Food and Drug Administration (FDA) and contains scientific and technical information about the major pathogens that cause these kinds of illnesses. A separate "consumer box" in each chapter provides non-technical information, in everyday language. The boxes describe plainly what can make you sick and, more important, how to prevent it.

Most foodborne illnesses, while unpleasant, go away by themselves and don't have lasting effects. But you'll read about some pathogens that can be more serious, have long-lasting effects, or cause death. To put these pathogens in perspective, think about how many different foods and how many times you eat each day, all year, without getting sick from the food. The FDA and other Federal agencies work together and with the food industry to make the U.S. food supply one of the safest in the world.

You also play a part in the safety of what you eat. When you read the consumer boxes, you'll see that different pathogens can be risky in different ways, and that a safety step that's effective against one might not be as effective against another. So what should you do? The answer is to follow some simple steps that, together, lower the risk from most pathogens.

- Washing your hands before and after handling food, and in between handling different foods, is one of the most important steps you can take. Do the same with equipment, utensils, and countertops.
- Wash raw fruits and vegetables under running water. These nutritious foods usually are safe, as you probably know from the many times you've eaten them, but wash them just in case they've somehow become contaminated. For the most part, the less of a pathogen on a food – if any – the less chance that it can make you sick.
- Cooking food to proper temperatures kills most bacteria, including *Salmonella*, *Listeria*, and the kinds of *E. coli* that cause illness, and parasites.
- Keep any pathogens that could be on raw, unwashed foods from spreading by keeping raw and cooked foods separate. Keep them in different containers, and don't use the same equipment on them, unless the equipment is washed properly in between. Treat countertops the same way.
- Refrigerate food at 40°F as soon as possible after it's cooked. Remember, the less of a pathogen there is in a food, the less chance that it can make you sick. Proper refrigeration keeps most types of bacteria from growing to numbers that can cause illness (although if a food already has high numbers of bacteria when it's put in the refrigerator, it could still cause illness).

Here are a few examples of why following *all* of these steps is important. Some types of bacteria form spores that aren't killed by cooking. Spores are a survival mode in which those bacteria make an inactive form that can live without nutrition and that develops very tough protection against the outside world. After cooking, the spores may change and grow into bacteria, when the food cools down. Refrigerating food quickly after cooking can help keep the bacteria from multiplying. On the other hand, cooking does kill most harmful bacteria. Cooking is especially important when a pathogen is hard to wash off of a particular kind of food, or if a bacterium can grow at refrigerator temperatures, as is true of *Listeria monocytogenes* and *Yersinia enterocolitica*.

As you read about the differences among the pathogens, remember that there's a common theme: following *all* the safety steps above can help protect you. The exceptions are toxins, such as the poisons in some mushrooms and a few kinds of fish and shellfish. Cooking, freezing, and washing won't necessarily destroy toxins. Avoiding them is your best protection, as you'll see when you read the chapters.

Authorship

The second edition of the Bad Bug Book would not have been possible without the contributions of the many FDA scientists who donated their time and expertise to update the chapters. The result of their efforts is a handbook that can serve as a valuable tool for food-safety professionals and others with an interest in food safety.

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Acknowledgments

The second edition of the Bad Bug Book would not have been possible without the contributions of the many FDA scientists who donated their time and expertise to update the chapters. The result of their efforts is a handbook that can serve as a valuable tool for food-safety professionals and others with an interest in food safety. Our gratitude is extended to Drs. Mickey Parish and Fred S. Fry Jr., for the insight they offered in their expert reviews of the book. The first edition of the Bad Bug Book was the concept of Dr. Mark Walderhaug, who executed it with the help of the many scientists working with him at the time, and the field is indebted to him and to them for their vision.

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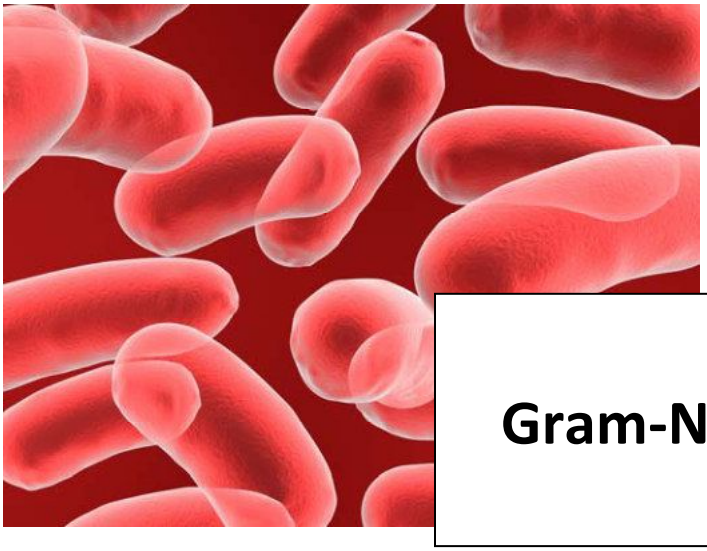

Gram-Negative Bacteria

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Foodborne Pathogenic Microorganisms and Natural Toxins

Salmonella species

1. Organism

Salmonella species (spp.)

Salmonella is a motile, non-sporeforming, Gram-negative, rod-shaped bacterium in the family *Enterobacteriaceae* and the tribe *Salmonellae*. Non-motile variants include *S.* Gallinarum and *S.* Pullorum. The genus *Salmonella* is divided into two species that can cause illness in humans:

- *S. enterica*
- *S. bongori*

Salmonella enterica, which is of the greatest public health concern, is comprised of six subspecies:

- *S. enterica* subsp. *enterica* (I)
- *S. enterica* subsp. *salamae* (II)
- *S. enterica* subsp. *arizonae* (IIIa)
- *S. enterica* subsp. *diarizonae* (IIIb)
- *S. enterica* subsp. *houtenae* (IV)
- *S. enterica* subsp. *indica* (VI)

Salmonella is further subdivided into serotypes, based on the Kaufmann-White typing scheme first published in 1934, which differentiates *Salmonella* strains by their surface and flagellar antigenic properties. *Salmonella* spp. are commonly referred to by their serotype names. For example, *Salmonella enterica* subsp. *enterica* is further divided into numerous serotypes, including *S.* Enteritidis and *S.* Typhimurium, which are common in the U.S. (Note that species names are italicized, but serotype names are not.) When Kaufmann first proposed the scheme, 44 serotypes had been discovered. As of 2007, the number of serotypes discovered was 2,579.

For Consumers: A Snapshot

Salmonella causes two kinds of illness:
(1) Gastrointestinal illness, which causes nausea, vomiting, diarrhea, cramps, and fever, with symptoms generally lasting a couple of days and tapering off within a week. In otherwise healthy people, the symptoms usually go away by themselves, but long-term arthritis may develop.
(2) Typhoidal illness causes high fever, diarrhea or constipation, aches, headache, and lethargy (drowsiness or sluggishness), and, sometimes, a rash. It's a very serious condition; up to 10% of people who don't get treatment may die. Many kinds of food can become contaminated with the first type, from meats and eggs to fruits and vegetables, and even dry foods, like spices and raw tree nuts. The typhoidal illness usually is associated with sewage-contaminated drinking water, or crops irrigated with sewage-contaminated water. Some pets, like turtles and other reptiles, and chicks, can carry *Salmonella*, which can spread to anything that comes into contact with the pet. For example, a pet owner can, through unwashed hands, contaminate foods or even his or her own face with *Salmonella*. This bacterium is hard to wash off of food, even with soapy water, so important measures for preventing foodborne illness from *Salmonella* include thorough cooking, hand washing, keeping raw foods separated from cooked foods, and keeping foods at the correct temperature (refrigerate foods at 40°F or below). In people with weak immune systems, *Salmonella* can spread to other organs and cause very serious illness.

2. Disease

Salmonella can cause two types of illness, depending on the serotype:

(1) nontyphoidal salmonellosis and (2) typhoid fever, both of which are described below. The symptoms of nontyphoidal salmonellosis can be quite unpleasant, but this illness is generally self-limiting among healthy people with intact immune systems (although it can cause life-threatening illness even in healthy people). Typhoid fever is more serious and has a higher mortality rate than does nontyphoidal salmonellosis.

Nontyphoidal Salmonellosis

- Caused by serotypes ***other than*** *S.* Typhi and *S.* Paratyphi A.
- **Mortality**: Generally less than 1%; however, *S*. Enteritidis has a 3.6% mortality rate in outbreaks in nursing homes and hospitals, with the elderly being particularly affected.
- **Onset**: 6 to 72 hours after exposure.
- **Infective dose**: As low as one cell, depending on age and health of host and strain differences among members of the genus.
- **Symptoms**: Nausea, vomiting, abdominal cramps, diarrhea, fever, headache.
- **Duration**: Symptoms generally last 4 to 7 days, with acute symptoms usually lasting 1 to 2 days or longer, depending on host factors, the dose ingested, and strain characteristics.
- **Complications**: (1) Dehydration and electrolyte imbalance may occur as a result of diarrhea and vomiting. This can lead to death in the very young, the elderly, and the immunocompromised, if not treated promptly. (2) In 2% of culture-proven cases, reactive arthritis (i.e., arthritis from an immune reaction to the infection – an autoimmune response – rather than directly from the infection itself) may follow 3 to 4 weeks after the onset of acute symptoms. Indications of reactive arthritis may include, for example, joint inflammation, urethritis, uveitis, and/or conjunctivitis. (3) Nontyphoidal *Salmonella* can sometimes escape from the gastrointestinal tract into the body and cause blood poisoning (septicemia) or infect the blood, internal organs, and/or joints (bacteremia). *S.* Dublin is sometimes associated with this complication.
- **Route of entry**: oral (e.g., ingestion of contaminated food, fecal particles, or contaminated water).
- **Pathway**: Penetration and passage of *Salmonella* organisms from gut lumen into epithelium of small intestine, where inflammation occurs. There is evidence that enterotoxin may be produced, perhaps within enterocytes.

Typhoid Fever

- Caused by serotypes *S.* Typhi and *S.* Paratyphi A, both of which are found only in humans.
- **Mortality**: Untreated, as high as 10%.

- **Onset**: Generally 1 to 3 weeks, but may be as long as 2 months after exposure.

- **Infective dose**: Fewer than 1,000 cells.

- **Symptoms**: High fever, from 103° to 104°F; lethargy; gastrointestinal symptoms, including abdominal pains and diarrhea or constipation; headache; achiness; loss of appetite. A rash of flat, rose-colored spots sometimes occurs.

- **Duration**: Generally 2 to 4 weeks.

- **Illness / Complications**: Septicemia, with colonization of other tissues and organs; e.g., may lead to endocarditis. Septic arthritis may occur, in which the infection directly affects the joints and may be difficult to treat. Chronic infection of the gallbladder may occur, which may cause the infected person to become a carrier.

- **Route of entry**: Oral (e.g., ingestion of contaminated food, fecal particles, or contaminated water).

- **Pathway**: Penetration and passage of typhoid *Salmonella* organisms from gut lumen into epithelium of small intestine and into the bloodstream (i.e., septicemia), which may carry the organisms to other sites in the body, where inflammation occurs. There is evidence that enterotoxin may be produced, perhaps within enterocytes.

3. Frequency of Disease

Annually in the United States:

- ***Nontyphoidal salmonellosis*** – A recent report from the Centers for Disease Control and Prevention (CDC) estimates that 1,027,561 cases of domestically acquired nontyphoidal salmonellosis occur annually in the U.S., when under-reporting and under-diagnosis are taken into account.

- ***Typhoid fever*** – In terms of domestically acquired *S. enterica* serotype Typhi, the same CDC report estimated that a mean of 1,821 cases occur annually in the U.S. Additional cases in the U.S. are associated with foreign travel. The report estimates that 433 cases of typhoid fever in the U.S., overall (i.e., whether or not they are domestically acquired), are culture-confirmed. The last case of a foodborne, noncarrier-based typhoid outbreak in the U.S. was in 1999 and was associated with the tropical fruit mamey.

4. Sources

Salmonella is widely dispersed in nature. It can colonize the intestinal tracts of vertebrates, including livestock, wildlife, domestic pets, and humans, and may also live in environments such as pond-water sediment. It is spread through the fecal-oral route and through contact with contaminated water. (Certain protozoa may act as a reservoir for the organism). It may, for example, contaminate meat, farm-irrigation water (thus contaminating produce in the field), soil and insects, factory equipment, hands, and kitchen surfaces and utensils.

Since *S.* Typhi and *S.* Paratyphi A are found only in human hosts, the usual sources of these organisms in the environment are drinking and/or irrigation water contaminated by untreated

sewage. It is highly recommended that only potable water and cooked vegetables be consumed in areas where these organisms are endemic.

Various *Salmonella* species have long been isolated from the outside of egg shells, but *S*. Enteritidis can be present inside the egg. This and other information strongly suggest vertical transmission; i.e., deposition of the organism on the albumen (egg white) side of the yolk-sack membrane (vitelline membrane) by an infected hen, prior to shell formation.

Outbreaks also have been linked to the handling of certain animals sometimes kept as pets, such as turtles, frogs, and chicks.

Food Sources

Although *Salmonella* traditionally was thought of as being associated with animal products in the past, fresh produce also has been the source of major outbreaks, particularly recently. The organism also survives well on low-moisture foods, such as spices, which have been the vehicles for large outbreaks.

A few examples of foods that have been linked to *Salmonella* illness include meats, poultry, eggs, milk and dairy products, fish, shrimp, spices, yeast, coconut, sauces, freshly prepared salad dressings made with unpasteurized eggs, cake mixes, cream-filled desserts and toppings that contain raw egg, dried gelatin, peanut butter, cocoa, produce (fruits and vegetables, such as tomatoes, peppers, and cantaloupes), and chocolate.

Cross Contamination

Cross contamination occurs when *Salmonella* is spread from a contaminated source – a contaminated food or an infected food handler or animal – to other foods or objects in the environment. An example of how this may occur is when potentially contaminated raw meats, poultry, seafood, produce, or eggs are not kept separate from each other during preparation or cooking, or when a food handler does not adequately clean utensils, surfaces, equipment, and hands after they have come into contact with these products.

The contamination can spread to factory and equipment surfaces, as well as kitchen surfaces and utensils. Cross contamination may occur at any point in the food process.

Cross contamination also may occur from handling pets or wildlife, such as turtles or frogs (or their water, soil, or food and water bowls), then handling food, food-preparation utensils, or other objects in the environment. (Even culinary frog legs have caused outbreaks of salmonellosis.)

5. Diagnosis

Serological identification of cultural isolates from stool. Genetic identification of approximately 100 *Salmonella* serotypes from pure culture is now possible, but the remaining 2,400-plus serotypes can be identified only through traditional serotyping.

6. Target Populations

Anyone, of any age, may become infected with *Salmonella*. Particularly vulnerable are people with weak immune systems, such as the very young and the elderly, people with HIV or chronic

illnesses, and people on some medications; for example, chemotherapy for cancer or the immunosuppressive drugs used to treat some types of arthritis. People with HIV are estimated to have salmonellosis at least 20 times more than does the general population and tend to have recurrent episodes.

7. Food Analysis

Isolation and detection methods have been developed for many foods having prior history of *Salmonella* contamination. Conventional culture and identification methods may require 4 to 6 days for presumptive results. To screen foods, several rapid methods are available, which require 1 to 2 days. These rapid methods include antibody and molecular (DNA or RNA) based assays, but in most cases, require a cultural means to confirm the presence of *Salmonella*, for regulatory purposes.

8. Examples of Outbreaks

For information on recent outbreaks, see the Morbidity and Mortality Weekly Reports from the Centers for Disease Control and Prevention (CDC).

9. Other Resources

- The CDC provides information about *Salmonella*, including information about preventing *Salmonella* Enteritidis infection, on avoiding salmonellosis from animal-handling, and on typhoid fever.
- Loci index for genome *Salmonella* Enteritidis is available from GenBank.

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Foodborne Pathogenic Microorganisms and Natural Toxins

Campylobacter jejuni

1. Organism

Campylobacter jejuni is a non-sporeforming, Gram-negative rod with a curved- to S-shaped morphology. Many strains display motility, which is associated with the presence of a flagellum at one or both of the polar ends of this bacterium.

Members of the *Campylobacter* genus are microaerophilic; i.e., they grow at lower-than-atmospheric oxygen concentrations. Most grow optimally at oxygen concentrations from 3% to 5%. Thus, these bacteria generally are fairly fragile in the ambient environment and somewhat difficult to culture in the laboratory. Additional conditions to which *C. jejuni* are susceptible include drying, heating, freezing, disinfectants, and acidic conditions.

Other *Campylobacter* species, such as *C. coli* and *C. fetus*, also cause foodborne diseases in humans; however, more than 80% of *Campylobacter* infections are caused by *C. jejuni*. *C. coli* and *C. jejuni* cause similar disease symptoms. *C. fetus* infections often are associated with animal contact or consumption of contaminated foods and beverages and are especially problematic for fetuses and neonates, in whom the mortality rate may be up to 70%.

For Consumers: A Snapshot

Campylobacter jejuni is estimated to be the third leading bacterial cause of foodborne illness in the U.S. (Certain viruses are the biggest known cause of foodborne illnesses, overall.) The symptoms this bacterium causes generally last from 2 to 10 days and, while the diarrhea (sometimes bloody), vomiting, and cramping are unpleasant, they usually go away by themselves in people who are otherwise healthy. Raw poultry, unpasteurized ("raw") milk and cheeses made from it, and contaminated water (for example, unchlorinated water, such as in streams and ponds) are major sources, but it also occurs in other kinds of meats and has been found in seafood and vegetables. Anyone can get sick from food contaminated with *Campylobacter*, but children younger than 5 years old and people 15 to 29 years old are more likely to get the infection than are others. Among these age groups, infants 6 to 12 months old have the highest rate of illness. People with weak immune systems also are at higher risk; for example, those with HIV/AIDS get sick from foodborne *Campylobacter* 40 times more often than do people in the same age group who have healthy immune systems. Very rarely, babies still in the womb have gotten the infection from their mothers, causing miscarriages or stillbirths. Overall, about 1 out of 1,000 people who get the infection die from it, but it happens rarely among otherwise healthy people. As with all bacteria that cause foodborne illness, consumers can take the following steps to help avoid *Campylobacter* infections: (1) **clean** raw vegetables and fruits, kitchen surfaces, utensils, and your hands; (2) **separate** raw foods from cooked foods, kitchen surfaces, utensils, and dinnerware, etc.; (3) **cook** raw foods according to instructions; (4) **refrigerate** foods, including leftover cooked foods, as soon as possible; and (5) use only pasteurized milk.

Campylobacter genomes are relatively unstable; several mechanisms that may lead to this genetic instability have been proposed, including bacteriophage activity, DNA recombination and transformation. There are several typing methods, such as pulsed-field gel electrophoresis, PCR-based typing, ribotyping and genomotyping, for assessing the genetic diversity of *C. jejuni*.

A list of *Campylobacter* genomes that have been sequenced is available under the National Center for Biotechnology Information web link.

2. Disease

- **Mortality**: The CDC attributes an estimated 76 deaths in the United States, per year, to campylobacteriosis.

- **Infective dose:** In general, the minimum number of ingested *Campylobacter* cells that can cause infection is thought to be about 10,000. However, in trials, as few as 500 ingested *Campylobacter* cells led to disease in volunteers. Differences in infectious dose likely can be attributed to several factors, such as the type of contaminated food consumed and the general health of the exposed person.

- **Onset**: The incubation period, from time of exposure to onset of symptoms, generally is 2 to 5 days.

- **Disease / complications:** The disease caused by *C. jejuni* infections is called campylobacteriosis. The most common manifestation of campylobacteriosis is self-limiting gastroenteritis, termed "*Campylobacter* enteritis," without need for antimicrobial therapy. When antimicrobial therapy is indicated, erythromycin or ciprofloxacin are most commonly prescribed.

  A small percentage of patients develop complications that may be severe. These include bacteremia and infection of various organ systems, such as meningitis, hepatitis, cholecystitis, and pancreatitis. An estimated 1.5 cases of bacteremia occur for every 1,000 case of gastroenteritis. Infections also may lead, although rarely, to miscarriage or neonatal sepsis.

  Autoimmune disorders are another potential long-term complication associated with campylobacteriosis; for example, Guillain-Barré syndrome (GBS). One case of GBS is estimated to develop per 2,000 *C. jejuni* infections, typically 2 to 3 weeks post infection. Not all cases of GBS appear to be associated with campylobacteriosis, but it is the factor most commonly identified prior to development of GBS. Various studies have shown that up to 40% of GBS patients first had *Campylobacter* infection. It is believed that antigens present on *C. jejuni* are similar to those in certain nervous tissues in humans, leading to the autoimmune reaction. Reactive arthritis is another potential long-term autoimmune complication. It can be triggered by various kinds of infections and occurs in about 2% of *C. jejuni* gastroenteritis cases.

  Hemolytic uremic syndrome and recurrent colitis following *C. jejuni* infection also have been documented.

- **Symptoms**: Fever, diarrhea, abdominal cramps, and vomiting are the major symptoms. The stool may be watery or sticky and may contain blood (sometimes occult – not discernible to the naked eye) and fecal leukocytes (white cells). Other symptoms often present include abdominal pain, nausea, headache, and muscle pain.

- **Duration**: Most cases of campylobacteriosis are self-limiting. The disease typically lasts from 2 to 10 days.

- **Route of entry**: Oral.

- **Pathway**: The mechanisms of pathogenesis by *C. jejuni* are not well understood and usually vary based on the virulence genes present in a particular strain. In general, C. *jejuni* cause infections by invading and colonizing the human gastrointestinal tract. Motility appears to be an important factor in *C. jejuni* pathogenesis, enabling the bacterium to invade the human intestinal mucosa. The mechanisms by which cellular invasion by *C. jejuni* cause the observed symptoms remain a mystery. In genome-sequencing studies, researchers were not able to identify the presence of toxin genes that likely contribute to diarrhea and other common symptoms.

3. Frequency

Campylobacter species are believed to be the third leading cause of domestically acquired bacterial foodborne illness in the United States, with an estimated 845,024 cases occurring annually, according to a 2011 Centers for Disease Control and Prevention (CDC) report. According to data from FoodNet, the incidence of cases of campylobacteriosis reported to the CDC in 2008 was 12.68 per 100,000 individuals, which is a decrease of 32% over the last decade. For each reported case of campylobacteriosis, it is estimated that 30 cases are unreported.

4. Sources

Major food sources linked to *C. jejuni* infections include improperly handled or undercooked poultry products, unpasteurized ("raw") milk and cheeses made from unpasteurized milk, and contaminated water. *Campylobacter* infection in humans has been linked to handling and eating raw or undercooked meat and poultry, whether fresh or frozen. Avoiding cross contamination of uncooked items from raw meat and poultry products, thorough cooking, pasteurization of milk and dairy products, and water disinfection are effective ways to limit food- and water-borne exposure to *Campylobacter.* Reduction of risk from contaminated poultry products can be achieved through good hygienic practices by manufacturers and consumers.

Campylobacter is part of the natural gut microflora of most food-producing animals, such as chickens, turkeys, swine, cattle, and sheep. Typically, each contaminated poultry carcass can carry 100 to 100,000 *Campylobacter* cells. Given the fact that up to 500 *Campylobacter* cells can cause infection, poultry products pose a significant risk for consumers who mishandle fresh or processed poultry during preparation or who undercook it.

C. *jejuni* has been found in a variety of other foods, such as vegetables and seafood, and in non-food animal species. *C. jejuni* also occurs in nonchlorinated water, such as that found in ponds and streams.

5. Diagnosis

Special incubation conditions are required for isolation and growth of *C. jejuni* cells, since the organism is microaerophilic. Samples from stool or rectal swabs are inoculated directly onto selective media, or they can be enriched to increase recovery. To limit growth of competing organisms, media used for cultivation usually are supplemented with blood and antimicrobial agents. The cultures are incubated at 42ºC, under microaerophilic conditions (5% oxygen and 5% to 10% carbon dioxide), for optimal recovery.

6. Target Populations

Children younger than 5 years old and young adults 15 to 29 years old are the populations in whom *C. jejuni* gastroenteritis most commonly is detected. The highest incidence of infection is among infants 6 to 12 months old. *C. jejuni* bacteremia may also affect pregnant women, leading to infection of the fetus, which can lead to miscarriage or stillbirth. The incidence of infection is estimated to be 40-fold greater in people with HIV/AIDS, compared with others in the same age group.

7. Food Analysis

Isolation of *C. jejuni* from food is difficult, because the bacteria are usually present in very low numbers. For isolation from most food products, samples are rinsed and the rinsate is collected and subjected to pre-enrichment and enrichment steps, followed by isolation of *C. jejuni* from the agar medium. For more information about isolation of *Campylobacter* from food and water, see FDA's Bacteriological Analytical Manual.

8. Examples of Outbreaks

For an update on recent outbreaks related to *Campylobacter*, please visit the CDC's Morbidity and Mortality Weekly Report.

The following reports are available on the surveillance of foodborne outbreaks in the U.S.: CDC annual report, CDC report #1, CDC report #2, and FoodNet report.

9. Other Resources

The following web links provide more information about *Campylobacter* and its prevention and control:

- U.S. Department of Agriculture – Q&A from Food Safety and Inspection Services
- A 1999 CDC article provides history and perspective. Altekruse SF, Stern NJ, Fields PI, Swerdlow DL. *Campylobacter jejuni*: An Emerging Foodborne Pathogen. Emerging Infectious Diseases, [serial on the Internet]. 1999, Feb.
- Several federal surveillance and monitoring programs in the U.S. report the incidences of *Campylobacter* infections and their resistance to antimicrobial drugs; for example, FoodNet, PulseNet, and National Antimicrobial Resistance Monitoring System.

Additional resources include:

- National Center for Biotechnology Information (taxonomy)
- World Health Organization
- FDA report on risk assessment

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Yersinia enterocolitica

Yersinia enterocolitica* and *Yersinia pseudotuberculosis

1. Organism

The *Yersinia* genus has 11 species; 4 are pathogenic, but only *Y. enterocolitica* and *Y. pseudotuberculosis* cause gastroenteritis. *Y. enterocolitica* and *Y. pseudotuberculosis* are small, rod-shaped, Gram-negative bacteria. The former is often isolated from clinical specimens, such as wounds, feces, sputum, and mesenteric lymph nodes. However, it is not part of the normal human flora. *Y. pseudotuberculosis* has been isolated from diseased human appendix. Both pathogens are transmitted through the fecal-oral route.

Both of these gastroenteritis-causing species have been isolated from animals, such as pigs, birds, beavers, cats, and dogs, and, in the case of *Y. enterocolitica*, frogs, flies, and fleas. *Y. enterocolitica* has been detected in environmental sources, such as soil and water (e.g., ponds and lakes). Most isolates are not pathogenic.

Y. enterocolitica is psychrotrophic (i.e., a microorganism that grows well at low temperature) and has the ability to grow at temperatures below 4°C. The doubling time, at 30°C, is 34 min; at 22°C, is 1 hr; and at 7°C, is 5 hrs. It can withstand freezing and can survive in frozen foods for extended periods. In fact, *Y. enterocolitica* has survived better in artificially contaminated food stored at room and refrigeration temperatures than at an intermediate temperature. It persists longer in cooked foods than in raw foods, due to increased nutrient availability.

For Consumers: A Snapshot

Food and water contaminated with this bacterium, *Yersinia*, can make people sick. Among the foods that have been linked to illness from *Yersinia* are pork (including chitterlings, sometimes called "chitlins"), unpasteurized milk, and oysters. (Pasteurized milk has been heated in a way that kills bacteria, but unpasteurized – "raw" – milk has not and is much riskier.) The illness, yersiniosis, also can be passed from contaminated hands into the mouth to cause the illness; for example, if an infected person doesn't wash his or her hands well after having a bowel movement and contaminates things that other people handle before touching their mouths or food. Anyone can get yersiniosis, but young children most often get it. The symptoms start within 1 day to 2 weeks, or even longer, and include high fever and stomach pain, with diarrhea and, sometimes, vomiting. The diarrhea may or may not be bloody. Besides young children, people who are elderly or in poor health or who have weak immune systems, or are on medications that weaken the immune system, are at highest risk. Some people get arthritis-like symptoms, such as joint pains and rashes (which often go away in a month or several months), or other, more serious complications that may affect the heart, for example. Most mild cases of yersiniosis go away by themselves, but health professionals can prescribe antibiotics to treat it, if necessary. To help protect yourself, follow basic food-safety tips, which include good hygiene, washing raw fruits and vegetables and the things they touch, cooking food well and keeping it apart from raw food, keeping food refrigerated at 40°F or lower, using pasteurized milk instead of "raw" milk, and using products made from pasteurized milk, ***not*** raw milk.

Y. enterocolitica can grow easily at refrigeration temperature in vacuum-packed meat, boiled eggs, boiled fish, pasteurized liquid eggs, pasteurized whole milk, cottage cheese, and tofu. Growth of the microorganism also occurs in refrigerated seafood – oysters, raw shrimp, and cooked crab meat. *Y. enterocolitica* and *Y. pseudotuberculosis* can grow over a pH range of 4 to 10, generally with an optimum pH of 7.6. They tolerate alkaline conditions very well, compared with acid conditions (although that depends on the kind of acid used, environmental temperature, composition of the medium, and growth phase of the bacteria).

Y. pestis, the causative agent of the plague, is genetically very similar to *Y. pseudotuberculosis*, but infects humans by routes other than food; e.g., fleas or aerosols. *Y. enterocolitica* has between 10% and 30% DNA homology with the Enterobacteriaceae family and is 50% related to *Y. pseudotuberculosis* and *Y. pestis*. Genetic analysis of *Y. pestis* revealed it to be a clone of *Y. pseudotuberculosis*, which evolved sometime between 1,500 to 20,000 years ago.

2. Disease

- **Mortality**: Fatalities are extremely rare.

- **Infective dose:** The medium infective dose for humans is not known, but is estimated to be between 10^4 to 10^6 organisms. The infective dose and clinical presentation of symptoms may depend on pathogen (strain-dependent) and host factors. For example, in some cases, in people with gastric hypoacidity, the infective dose may be lower.

- **Onset**: Incubation times from 1 to 11 days have been observed, but occasionally last for several months.

- **Illness / complications**: In some patients, complications arise due to the strain type causing the initial infection and specific human immunologic leukocyte antigen, HLA-B27. These sequelae include reactive arthritis; glomerulonephritis; endocarditis; erythema nodosum (which occurs predominantly in women); uveitis; thyroid disorders, such as Graves' disease; hyperthyroidism; nontoxic goiter; and Hashimoto's thyroiditis. *Y. enterocolitica* has been associated with reactive arthritis, which may occur even in the absence of obvious symptoms. The frequency of such postenteritis arthritic conditions is about 2% to 3%. In Japan, *Y. pseudotuberculosis* was implicated in the etiology of Kawasaki's disease.

  Another complication is bacteremia, which raises the possibility of disease dissemination. However, this is rare. Performance of unnecessary appendectomies also may be considered a major complication of yersiniosis, as one of the main symptoms of the disease is abdominal pain in the lower right quadrant.

  Treatment includes supportive care, since the gastroenteritis is self-limiting. If septicemia or other invasive diseases occur, antibiotic therapy with gentamicin or cefotaxime (doxycycline and ciprofloxacin) typically are administered.

- **Symptoms:** Infection with *Y. enterocolitica* manifests as nonspecific, self-limiting diarrhea, but may cause a variety of autoimmune complications, as noted above. Most symptomatic infections occur in children younger than 5 years old. Yersiniosis in these children is frequently characterized as gastroenteritis, with diarrhea and/or vomiting; however, fever and abdominal pain are the hallmark symptoms. A small proportion of

children (less than 10%) produce bloody stools. Children usually complain of abdominal pain and headache and sore throat at the onset of the illness.

Yersinia infections mimic appendicitis and mesenteric lymphadenitis, but the bacteria may also cause infection in other sites, such as wounds, joints, and the urinary tract.

- **Duration**: The illness might last from a few days to 3 weeks, unless it becomes chronic enterocolitis, in which case it might continue for several months.

- **Route of entry**: Oral.

- **Pathway**: As zoonotic pathogens, *Y. enterocolitica* and *Y. pseudotuberculosis* enter the gastrointestinal tract after ingestion of contaminated food or water. Gastric acid is a significant barrier to infection. The infective dose might be lower among people with gastric hypoacidity. Both pathogens harbor plasmid (pYV)-encoded virulence genes that affect pathogenesis. These include an outer-membrane protein, YadA (*Yersinia* adhesion A), and the genetic suite comprising the type III secretory system. This process usually is facilitated by Yops proteins, which contribute to the ability of *Y. enterocolitica* cells to resist phagocytosis by causing disruption (cytotoxic changes) of mammalian (human) cells.

3. Frequency

Yersiniosis is far more common in Northern Europe, Scandinavia, and Japan than in the United States. It does not occur frequently and tends to be associated with improper food-processing techniques. *Y. enterocolitica* is a more frequent cause of yersiniosis than is *Y. pseudotuberculosis*, and cases have been reported on all continents. Different biotypes of *Y. enterocolitica* have been associated with infections around the world, with the most common biotype being 4/O:3. Information on *Y. pseudotuberculosis* is not as well defined and, as such, is reported less frequently than is *Y. enterocolitica*.

4. Sources

Strains of *Y. enterocolitica* can be found in meats (pork, beef, lamb, etc.), oysters, fish, crabs, and raw milk. However, the prevalence of this organism in soil, water, and animals, such as beavers, pigs, and squirrels, offers many opportunities for *Yersinia* to enter the food supply. For example, poor sanitation and improper sterilization techniques by food handlers, including improper storage, may be a source of contamination. Raw or undercooked pork products have drawn much attention as a source of *Y. enterocolitica*, and *Y. pseudotuberculosis*, particularly since *Y. enterocolitica* has been associated with pigs.

5. Diagnosis

Yersiniosis may be misdiagnosed as Crohn's disease (regional enteritis) or appendicitis. Diagnosis of yersiniosis begins with isolation of the organism from the human host's feces, blood, or vomit, and sometimes at the time of appendectomy. Confirmation occurs with the isolation, as well as biochemical and serological identification, of *Y. enterocolitica* from both the human host and the ingested food. Diarrhea occurs in about 80% of cases; abdominal pain and fever are the most reliable symptoms.

Y. enterocolitica or *Y. pseudotuberculosis* in patients with acute gastroenteritis can be readily isolated via conventional bacteriological media designed to isolate *Yersinia*. It is much more challenging to isolate these pathogens in asymptomatic carriers or from foods. Since many *Y. enterocolitica* isolated from non-human sources are not considered pathogenic, it is imperative to distinguish these isolates from pathogenic *Yersinia* species. Molecular-based assays, particularly PCR methods, have been developed to target *Y. enterocolitica* and can be used to rapidly confirm the pathogenicity of the isolate. Several PCR primer sets are directed to either plasmid-borne genes, e.g., *virF* or *yadA*, or chromosomally located loci, such as *ail*.

Serology is used to identify the biotype (based on biochemical analysis) and serogroup (O-antigen). Sera from acute or convalescent patients are titered against the suspect serotype of *Yersinia* spp.

6. Target populations

The most susceptible populations for the main disease and potential complications are the very young (< 10 years), the debilitated, the very old, and people undergoing immunosuppressive therapy. Those most susceptible to post-enteritis arthritis are people with the antigen HLA-B27 (or related antigens, such as B7).

7. Food Analysis

The isolation method is relatively easy to perform, but in some instances, cold enrichment (25 g sample of the food mixed with 225 ml of Peptone Sorbitol bile broth for 10 days at 10°C) may be required. *Y. enterocolitica* can be presumptively identified in 36 to 48 hours using biochemical testing or API 20E or Vitek GNI. The genes encoding for invasion of mammalian cells are located on the chromosome, while a 70 kb plasmid, present in almost all pathogenic *Yersinia* species, encodes most of the other virulence-associated phenotypes. PCR-based assays have been developed to target virulence genes on both the chromosome and plasmid**.**

8. Examples of Outbreaks

To date, no foodborne outbreaks caused by *Y. pseudotuberculosis* have been reported in the U.S., but human infections transmitted via contaminated water and foods have been reported in Japan (Fukushima *et al*. 1988) and Finland (Jalava *et al*. 2004). *Y. pseudotuberculosis* has been implicated in a number of food-related outbreaks, but the number of foodborne outbreaks from *Y. enterocolitica* is higher.

For more information about recent outbreaks, see the Morbidity and Mortality Weekly Reports from CDC.

9. Resources

- Loci index for genome *Yersinia enterocolitica* and
  Loci index for genome *Yersinia pseudotuberculosis* are available from GenBank.
- Robins-Browne, R. (2007). *Food Microbiology: Fundamentals and Frontiers*, 3rd ed. American Society for Microbiology Press, Washington, D. C.

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Shigella species

Shigella sonnei*, *S. boydii*, *S. flexneri*, and *S. dysenteriae

1. Organism

Shigellae are Gram-negative, non-motile, non-sporeforming, rod-shaped bacteria. *Shigella* species, which include *Shigella sonnei*, *S. boydii*, *S. flexneri*, and *S. dysenteriae*, are highly infectious agents. Some strains produce enterotoxins and Shiga toxin. The latter is very similar to the toxins produced by *E. coli* O157:H7.

Humans are the only host of *Shigella*, but it has also been isolated from higher primates. The organism is frequently found in water polluted with human feces.

In terms of survival, shigellae are very sensitive to environmental conditions and die rapidly. They are heat sensitive and do not survive pasteurization and cooking temperatures. In terms of growth, shigellae are not particularly fastidious in their requirements and, in most cases, the organisms are routinely cultivated in the laboratory, on artificial media. However, as noted in subsequent sections, the relative difficulty of cultivating this organism is dependent, in part, on the amount of time within which stool or food samples are collected and processed.

Shigella species are tolerant to low pH and are able to transit the harsh environment of the stomach. These pathogens are able to survive and, in some cases, grow in foods with low pH, such as some fruits and vegetables. They are able to survive on produce commodities packaged under vacuum or modified atmosphere and can also survive in water, with a slight decrease in numbers.

For Consumers: A Snapshot

Shigella is a bacterium that spreads from contaminated feces. It often spreads through unclean water, whether it's drinking water or swimming-pool water that an infected person has been in, even though the water might look clean. Food can become contaminated if it's handled by an infected person who didn't wash his or her hands well after having a bowel movement, or if contaminated water is used for growing fruits or vegetables or to rinse them afterwards. It doesn't take much *Shigella* to cause illness, and tiny bits of feces also can pass from the unwashed hands of an infected person (even though they might not look dirty) onto the hands and into the mouth of another person, causing that person to become sick. Although the illness it causes, shigellosis, often is mild and goes away by itself in about a week or less, it can become very serious in some cases. In those cases, there may be so much diarrhea (dysentery) that the body loses dangerous amounts of fluids and certain minerals, and it could lead to death. These people, especially, should see a health professional. Severe cases can be treated with certain antibiotics. Mild cases usually are not treated with antibiotics. Young children, the elderly, and people with a weak immune system, such as people with HIV/AIDS, are more likely than others to develop severe illness. Whether mild or severe, the illness usually starts within 8 hours or up to about 2 days. The diarrhea is often bloody and may contain pus or mucus, and there may be vomiting, cramps, and fever. Good handwashing after going to the bathroom is one of the most important food-safety tips for protecting yourself and others from *Shigella*. Following cooking directions on food packages also can help protect you, because proper cooking kills *Shigella*.

2. Disease

The illness caused by *Shigella* is shigellosis (also called bacillary dysentery), in which diarrhea may range from watery stool to severe, life-threatening dysentery. All *Shigella* spp. can cause acute, bloody diarrhea. *Shigella* spp. can spread rapidly through a population, particularly in crowded and unsanitary conditions.

S. dysenteriae type 1 causes the most severe disease and is the only serotype that produces the Shiga toxin, which may be partially responsible for cases in which hemolytic uremic syndrome (HUS) develops. *S. sonnei* produces the mildest form of shigellosis; usually watery diarrhea. *S. flexneri* and *S. boydii* infections can be either mild or severe.

In developed countries, *S. sonnei* is the *Shigella* species most often isolated, whereas *S. flexneri* predominates in developing countries.

- **Mortality**: In otherwise healthy people, the disease usually is self-limiting, although some strains are associated with fatality rates as high as 10-15%. (See Illness / complications section, below.)

- **Infective dose:** As few as 10 to 200 cells can cause disease, depending on the age and condition of the host.

- **Onset**: Eight to 50 hours.

- **Illness / complications:** In otherwise healthy people, the disease usually consists of self-limiting diarrhea (often bloody), fever, and stomach cramps. Severe cases, which tend to occur primarily in immunocompromised or elderly people and young children, are associated with mucosal ulceration, rectal bleeding, and potentially drastic dehydration. Potential sequelae of shigellosis include reactive arthritis and hemolytic uremic syndrome.

- **Symptoms**: May include abdominal pain; cramps; diarrhea; fever; vomiting; blood, pus, or mucus in stools; tenesmus (straining during bowel movements).

- **Duration**: Uncomplicated cases usually resolve in 5 to 7 days. Most of the time, the illness is self-limiting. In some circumstances, antibiotics are given; usually trimethoprim-sulfamethoxazole, ceftriaxone, or ciprofloxacin.

- **Route of entry**: The fecal-oral route is the primary means of human-to-human transmission of *Shigella*. With regard to foods, contamination is often due to an infected food handler with poor personal hygiene.

- **Pathway**: The disease is caused when *Shigella* cells attach to, and penetrate, colonic epithelial cells of the intestinal mucosa. After invasion, they multiply intracellularly and spread to contiguous epithelial cells, resulting in tissue destruction. As noted, some strains produce enterotoxin and Shiga toxin similar to those produced by *E. coli* O157:H7.

3. Frequency

A recent Centers for Disease Control and Prevention (CDC) report on domestically acquired foodborne illnesses in the United States revealed that about 15,000 laboratory-confirmed isolates are reported each year, with estimates of actual occurrence ranging from 24,511 to 374,789 cases (average of 131,243). About 31% of these are estimated to be foodborne. Estimates of foodborne illness episodes (mean) caused by 31 pathogens placed *Shigella* as the sixth most frequent cause (after norovirus, *Salmonella* species, *Clostridium perfringens*, *Campylobacter*, and *Staphylococcus aureus*, in that order).

Episodes of shigellosis appear to follow seasonal variations. In developed countries, the highest incidences generally occur during the warmer months of the year.

4. Sources

Most cases of shigellosis are caused by ingestion of fecally contaminated food or water. In the case of food, the major factor for contamination often is poor personal hygiene among food handlers. From infected carriers, this pathogen can spread by several routes, including food, fingers, feces, flies, and fomites.

Shigella is commonly transmitted by foods consumed raw; for example, lettuce, or as non-processed ingredients, such as those in a five-layer bean dip. Salads (potato, tuna, shrimp, macaroni, and chicken), milk and dairy products, and poultry also are among the foods that have been associated with shigellosis.

5. Diagnosis

Diagnosis is by serological or molecular identification of cultures isolated from stool. *Shigella* may be more difficult to cultivate if stool samples are not processed within a few hours.

6. Target Populations

All people are susceptible to shigellosis, to some degree, but children 1 to 4 years old, the elderly, and the immunocompromised are most at risk. Shigellosis is very common among people with AIDS and AIDS-related complex.

7. Food Analysis

Shigellae remain a challenge to isolate from foods. A molecular-based method (PCR) that targets a multi-copy virulence gene has been developed and implemented by FDA. Improvements in the bacterial isolation method continue and should be available in the near future.

The window for collecting and processing *Shigella* from foods, for cultivation, may be days (rather than hours, as is the case with stool), depending on the food matrix and storage conditions; e.g., temperature. *Shigella* species can be outgrown by the resident bacterial populations found in foods, which may reflect the usual low numbers of the organism present in foods and, in some foods, a very large number of non-*Shigella* bacteria. Another factor that reduces the chance of isolating *Shigella* from foods may be the physiological state of the pathogen at the time of analysis. Environmental conditions could affect its ability to either grow or survive in any food matrix.

8. Examples of Outbreaks

The CDC's Morbidity and Mortality Weekly Reports provide information about *Shigella* outbreaks.

9. Other Resources

Loci index for genome *Shigella* spp.

GenBank Taxonomy database

More information about *Shigella* and shigellosis can be found on the CDC website.

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Vibrio parahaemolyticus

1. Organism

This bacterium is a Gram-negative, curve-shaped rod frequently isolated from the estuarine and marine environments of the United States and other tropical-to-temperate coastal areas, worldwide. Both pathogenic and non-pathogenic forms of the organism can be isolated from marine and estuarine environments and from seafood harvested from these environments.

In general, the majority of *V. parahaemolyticus* isolates from the environment are non-pathogenic. Currently, pathogenic strains are identified by the presence of one or both of the hemolysins TDH (thermostable direct hemolysin) and TRH (thermostable-related hemolysin).

Optimal temperatures for *V. parahaemolyticus* are 20°C to 35°C; it can grow at temperatures up to 41°C. It is slowly inactivated at temperatures <10°C (minimum growth temperature), and cultures should never be stored in refrigerators. *V. parahaemolyticus* is halophilic; the highest abundance in oysters is at 23 ppt salt. It is lysed almost immediately in freshwater; thus, it is not usually transmitted via the fecal-oral route. At least 0.5% NaCl is required in all media, and 2% NaCl is optimal. Like other vibrios, *V. parahaemolyticus* is highly susceptible to low pH, freezing, and cooking. Most strains of *V. parahaemolyticus* produce a capsule, but all strains can be killed by common disinfectants, such as bleach and alcohol.

For Consumers: A Snapshot

There are different kinds of *Vibrio*, a bacterium that can cause illness when contaminated seafood is eaten. Illness from this kind of *Vibrio* is linked mostly to oysters, although other kinds of contaminated fish and shellfish also sometimes cause the illness. It doesn't cause cholera (that kind of *Vibrio* is covered in another chapter), but can cause bloody diarrhea, stomach cramps, fever, nausea, and/or vomiting, which usually are fairly mild and last less than a week. But in people with weak immune systems, it can spread to the blood and cause serious or deadly infections in other parts of the body. Examples of people at higher risk are those with diabetes, liver disease, kidney disease, cancer, AIDS, or other illnesses that weaken the immune system, and those on medications meant to lower the actions of the immune system, like some kinds of drugs for rheumatoid arthritis or cancer treatment. These people, especially, should always thoroughly cook their seafood, and should see a health professional if they develop symptoms. This kind of *Vibrio* usually lives in ocean water along the coast or in estuaries where, for example, ocean water comes together with river water. Water contaminated with *Vibrio* can cause illness if people drink the water or eat seafood that has been living in it, or if the contaminated water comes into contact with food in other ways. You can help protect yourself by cooking seafood until the inside reaches a temperature, for at least 15 seconds, of 145°F, but 155°F for things like fishcakes and 165°F for stuffed fish. Because bacteria, such as *Vibrio*, can grow in foods that have been cooked, but have then been contaminated by raw food, be sure to keep raw foods from touching cooked foods and surfaces used for cooking or eating. It's also important to wash raw foods in sanitary water and wash hands, equipment, and cooking and food-handling surfaces; and keep food refrigerated at 40°F or lower. After kitchen surfaces are washed, sanitize them with a commercially available product that's sold as a kitchen sanitizer. You might have heard people say that you should eat oysters or other shellfish only in months with the letter "R" – for example Janu**a**r**y**, Feb**r**u**a**r**y**, etc. But remember that *Vibrio* and other bacteria (and viruses) that affect seafood can cause illness in *any* month, so follow basic food-safety tips all year long.

2. Disease

(Note: *Vibrio parahaemolyticus* does not cause cholera and should not be confused with *Vibrio* species that do; i.e., *Vibrio cholerae*, which are addressed in a separate chapter)**.**

- **Mortality**: Death occurs in approximately 2% of gastroenteritis and 20% to 30% of septicemia cases.

- **Infective dose**: The FDA *V. parahaemolyticus* Risk Assessment states that the ID50 (median infective dose) is 100 million organisms. However, evidence from an outbreak in 2004 suggests an infectious dose >1,000-fold less than in the FDA risk assessment.

- **Onset**: The incubation period is 4 to 90 hours after ingestion of the organism, with a mean of 17 hours.

- **Illness / complications**: *V. parahaemolyticus*-associated gastroenteritis is the name of the infection caused by consumption of this organism. It is usually mild or moderate. Diarrhea caused by this organism is usually self-limiting, with less than 40% of reported cases requiring hospitalization and/or antibiotic treatment.

  Although the illness is generally mild or moderate, *V. parahaemolyticus* can also cause septicemia in susceptible people. Those at risk include people with diabetes, liver disease, kidney disease, cancer, AIDS, or other illnesses that result in an immunocompromised state, and those on immunosuppressive medications.

  In addition to the foodborne gastrointestinal illness, this organism also can cause wound infections. This occurs either through exposure of a pre-existing wound to contaminated marine or estuarine water or through wounds incurred while handling fish, shellfish, or crustaceans.

- **Symptoms**: Diarrhea, abdominal cramps, nausea, vomiting, fever, and bloody diarrhea may be associated with gastroenteritis infections caused by this organism.

- **Duration**: The median duration of the illness is 2 to 6 days.

- **Route of entry**: Oral (in the case of foodborne, gastroenteritis infections. As noted, wound infections also can occur through direct exposure).

- **Pathway**: The complete pathway by which *V. parahaemolyticus* causes disease remains unclear. However, it is known that TDH is a pore-forming toxin that lyses red blood cells and can attack intestinal cells, disrupting the electrolyte balance. The mechanism of TRH toxin is similar to TDH, disrupting electrolyte flux in intestinal cells.

3. Frequency

The Centers for Disease Control and Prevention (CDC) estimates that about 45,000 illnesses from *V. parahaemolyticus* occur each year, in the United States, and that about 86% of them are foodborne. A correlation exists between probability of infection and warmer months, when water temperatures are greater than 15°C (59°F). CDC estimates that only 1 in 20 cases of *V. parahaemolyticus* are reported, and it is likely that hospitalization and death are rare among unreported cases.

4. Sources

In the U.S., infections with this organism generally are associated with consumption of raw or improperly cooked oysters. Other seafood products, including finfish, squid, octopus, lobster, shrimp, crab, and clams, have been linked to *V. parahaemolyticus* illnesses, more frequently in Asian countries.

Thorough cooking kills the *Vibrio* organisms, so illnesses usually occur from consumption of raw seafood or cooked seafood that has been contaminated with raw product. Improper refrigeration of seafood products contaminated with this organism will allow its proliferation, which increases the possibility of infection.

5. Diagnosis

Diagnosis is made by culturing the organism from a person's stool, wound, or blood (in septicemia cases).

6. Target Populations

Anyone who eats raw or improperly cooked seafood products is susceptible to infection by this organism. People with compromised immune systems are at greater risk of septicemia and death.

7. Food Analysis

FDA's Bacteriological Analytical Manual (BAM) describes the methods most commonly used to isolate this organism from foods. Many food isolates are non-pathogenic; therefore, testing food isolates for the virulence determinants is recommended. The BAM recommends a DNA probe and/or a PCR procedure for identification of genes responsible for TDH and TRH production. Additionally, there are more recent molecular methods available for virulence characterization, many of which can be applied directly to seafood products, to screen for the presence of pathogenic organisms prior to isolation.

8. Examples of Outbreaks

Shellfish were linked to 177 cases in New York, Oregon, and Washington, in 2006. In 2004, in Alaska, 62 cases were linked to consumption of raw oysters. Reported outbreaks can be found in CDC's Morbidity and Mortality Weekly Reports.

9. Other Resources

The National Center for Biotechnology Information Taxonomy provides information about the historical classification of *V. parahaemolyticus*, as well as current genetic sequence information.

CDC provides information about *V. parahaemolyticus*.

The FDA risk assessment on *Vibrio parahaemolyticus* structures knowledge about *V. parahaemolyticus* in a systematic manner. It includes mathematical models developed to estimate exposure to this microorganism, dose-response relationships, and effectiveness of mitigation strategies.

Additional Reading

FDA. 2010. Quantitative risk assessment on the public health impact of pathogenic *Vibrio parahaemolyticus* in raw oysters. U.S. Food and Drug Administration, Washington, D.C.
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Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Coxiella burnetii

1. Organism

Coxiella burnetii, a Gram-negative, obligate intracellular bacterium, is the causative agent of Q fever. *Coxiella* are noted for their resistance to extremely harsh physical conditions, such as heat, low and high pH, and desiccation, due to a tough, spore-like stage. These cells may survive for long periods in the environment and in contaminated foods, such as unpasteurized milk. Due to *C. burnetii*'s ability to be disseminated via aerosols and its low infective dose, the Centers for Disease Control and Prevention (CDC) have declared it a Category B potential bioterrorism agent.

Coxiella burnetii has long been considered the most heat-resistant non-spore-forming pathogen found in milk, making it the benchmark organism for determining proper pasteurization conditions.

2. Disease

Q fever initially was described as a disease called "illness Q" (for "query") that occurred in Australian slaughterhouse (abattoir) workers in the late 1930s and is now known as a nearly worldwide zoonosis typically associated with livestock-handling occupations. There are two forms of the disease: (1) an acute form that may or may not be symptomatic and generally is less serious, although it has the potential for complications, and (2) a less common, chronic form that tends to be more severe and is associated with higher mortality rates.

For Consumers: A Snapshot

This bacterium causes a disease called Q fever, which can exist in two different forms. Some people don't have symptoms. Others do, and how sick they become depends on what form of the illness they have. (1) In one form, symptoms usually go away on their own (although they can become more serious) or with antibiotics. Symptoms may differ from person to person, but common ones are high fever; severe headaches; muscle aches; chills; heavy sweating; nausea, vomiting, or diarrhea; dry cough; and abdominal or chest pain. The fever usually lasts a week or two. With this form of the illness, about 1 percent of people die, when the infection spreads to the heart or lungs, for example. (2) The other, chronic form is more serious and can lead to death in more than 60 percent of untreated cases. Most people don't get this form; it usually occurs in people who have another serious illness or are pregnant. Chronic Q fever may not develop until 6 weeks or many years after a person is infected. The infection often spreads to the lining of the heart and can also spread to the brain or the lining of the brain or to the liver or lungs, for example. The disease comes back in about half of people with chronic Q fever who seemed to have recovered with treatment.

The most common way people become infected is by inhaling contaminated airborne particles; for example, in a slaughterhouse. But drinking **un**pasteurized milk or eating products that contain it also poses a risk. You can protect yourself by *not* drinking **un**pasteurized (raw) milk or eating food that contains it. Check food labels to make sure milk has been pasteurized, which means harmful bacteria have been killed.

- **Acute Q Fever**
  - **Mortality:** < 1%
  - **Infective dose:** Presumed to be fewer than 10 bacteria.
  - **Onset:** May occur as soon as 2 weeks after exposure; average time to symptoms is 20 days.

- **Illness / complications:** The infection sometimes is asymptomatic. Symptomatic individuals with acute Q fever experience a flu-like disease. Infections generally are easy to resolve with antibiotic treatment. Complications that may occur in more serious cases include pneumonia, hepatitis, and myocarditis.
- **Symptoms:** Symptoms may vary considerably among individuals, but commonly include very high fever (105°F); severe headaches; muscle aches; chills; profuse sweating; nausea, vomiting, and/or diarrhea; a dry cough; and abdominal and/or chest pain.
- **Duration:** The fever usually lasts 1 to 2 weeks and generally is self-limiting.
- **Route of entry:** Transmission of *C. burnetti* is primarily through inhalation of aerosolized bacteria, although transmission via ingestion of contaminated unpasteurized ("raw") milk or dairy products or via tick bite also is possible.
- **Pathway:** Once the bacteria enter host cells, the bacteria multiply in protective vacuoles before lysing the host cells and spreading to other, non-infected cells.

- **Chronic Q Fever**
  - **Mortality:** If untreated, may be > 60%.
  - **Infective dose:** See Acute Q Fever, above.
  - **Onset:** Chronic Q fever may develop 6 weeks to years after the acute illness.
  - **Illness / complications:** Less than 5% of infected patients will exhibit chronic Q fever. This more severe disease typically occurs in patients who are already compromised due to pregnancy, heart-valve disease, or other illness. A majority of cases involve endocarditis, but may also include hepatitis, encephalitis, pericarditis, meningitis, or pneumonia.
  - **Symptoms:** Symptoms depend on the tissue affected; e.g., symptoms of endocarditis, hepatitis, encephalitis, pericarditis, meningitis, or pneumonia.
  - **Duration:** Although often curable with extended (> 18 months) antibiotic treatment, 50% of patients are prone to relapses.
  - **Route of entry:** See Acute Q Fever, above.
  - **Pathway:** See Acute Q Fever, above.

3. Frequency

Since first becoming a reportable disease, the number of Q fever cases has steadily increased, from 17 cases with onset in 2000 to more than 160 cases with onset in 2007. In 2008, after CDC began recording cases based on type of Q fever, 132 cases, total, were reported, consisting of 117 acute and 15 chronic cases. Since that time, 90 to 110 acute and 20 to 25 chronic cases of Q fever have been reported each year.

4. Sources

C. burnetii is found nearly worldwide and is excreted in the urine, milk, feces, and birth products of its various hosts, which include humans, cattle, sheep, goats, reptiles, and birds. Ticks also are a reservoir and may transmit the bacteria directly, via bite, or indirectly, via infected feces. Inhalation of aerosolized bacteria is the most common route of transmission, although transfer

also may occur through ingestion of contaminated unpasteurized milk or dairy products and, as noted, via ticks.

5. Diagnosis

Q fever clinical diagnosis is difficult, due to the many different diseases it mimics. However, polymerase chain reaction (PCR) can be used for early detection of the disease when more conventional antibody tests are not useful. After full development of the disease, less-sensitive serological tests, such as an indirect immunofluorescence assay (IFA) against *C. burnetii*-specific antibodies, are capable of confirming the diagnosis.

6. Target populations

Q fever is associated most with occupations in the livestock industry, especially where aerosolization of livestock birth products may be common**.** Q fever is more common in males than in females and in adults more than in children, probably due to the occupational characteristics of livestock workers. The average age of affected individuals is 45 to 50 years old. Women are at risk of miscarriage if infected.

7. Food Analysis

The FDA-mandated level of pasteurization for milk sold in interstate commerce is lethal to *C. burnetii*, essentially obviating the need to detect the organism in this product. Were analysis for *C. burnetii* necessary, a live animal host or tissue culture would be required for propagation of the organism.

8. Examples of Recent Outbreaks

In July, 2011, three women, in Michigan (ages 30 to 40), were diagnosed with acute Q fever after drinking unpasteurized raw milk obtained as part of a herd-share arrangement.

In April 2011, in Washington state, an outbreak involving six illnesses occurred, presumed to have been caused by inhalation of barnyard dust particles contaminated by infected goats. Some of these goats were sold and were suspected of being the source of a Montana outbreak that included six cases.

An extremely large outbreak in the Netherlands caused nearly 4,000 illnesses over a 4-year span, starting in 2007. In this case, dairy goats and sheep appeared to be the sources of the outbreak, with 30 farms experiencing extremely high livestock abortion rates.

9. Other resources

FDA questions and answers about raw milk

Information about Q fever is available from CDC.

Recent *Coxiella* outbreaks, from CDC's Morbidity and Mortality Weekly Report.

NCBI Taxonomy Database

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Brucella species

1. Organism

Brucella spp. are small, Gram-negative, short, non-sporeforming coccobacilli. Members of the genus *Brucella*, of which there are six recognized species, belong to a class of Proteobacteria known as *Alphaproteobacteria.* Diverse groups of organisms comprise this class, including symbionts and plant pathogens, intracellular animal pathogens, and environmentally ubiquitous bacteria.

Strictly defined, *Brucella* spp. are facultative, intracellular parasites able to invade, and replicate in, phagocytes of the host and to multiply in bacteriologic media. CO_2-dependent *B. abortus* strains exist, and *B. ovis* grows only in atmospheres containing 5-10% CO_2. While evidence suggests that *Brucella* spp. can survive in the environment, it is less clear whether or not the bacteria can proliferate extensively outside the host.

Unlike other pathogenic bacteria, *Brucella* spp. do not possess plasmids or lysogenic bacteriophages, which accounts for the organism's relatively (but not entirely) static genome. Were *Brucella* to possess these factors, they would likely result in changes to the organism's pathogenicity, by enabling the organism to undergo more rapid exchange of genetic material or by introduction of the attacking bacteriophage's DNA into *Brucella*'s DNA, respectively.

For Consumers: A Snapshot

Brucella is a bacterium estimated to cause about 120 cases of confirmed human illness in the U.S. each year. It's carried by certain animals who often miscarry or abort their fetuses when first infected, but don't suffer other significant ill effects. They can transmit the bacterium to people, who could get sick with an illness called brucellosis; for example, to a farmer who helps an infected cow deliver a calf or to someone who drinks unpasteurized ("raw") milk that came from an infected cow. Transmission from human to human is rare. Livestock in the U.S. are part of brucellosis-free herds or are vaccinated against *Brucella*, so most human cases from **un**pasteurized milk or soft cheeses or other products made from it are usually linked to products that came from other countries. (But consumers beware: even the **un**pasteurized milk and milk products produced in the U.S. can carry other bacteria that cause serious illness or death, and the FDA strongly discourages consumers from drinking or eating unpasteurized milk products, regardless of where they're from.) Brucellosis, the disease caused by *Brucella*, is more common in developing countries. The disease also is called "undulant fever," because the high fevers and sweating that are characteristic of the illness come and go, and this may last for months or years. For this reason, the illness often is treated with a combination of antibiotics, and is treated for a longer time than is usual for most bacterial infections, preventing relapse in about 90% of cases. Although the death rate from *Brucella* infection is low in the U.S. – less than 2% – the disease can develop into serious or fatal complications; for example, it can infect the lining of the heart or the heart muscle itself, the brain and the layers covering it, the joints, or the spinal column.

Another property of *Brucella* species is their strong preference for a particular animal host, as follows (with hosts in parentheses): *B. melitensis* (sheep, goat), *B. abortus* (cattle), *B. suis* (pigs, hares, reindeer, wild rodents), *B. neotomae* (desert wood rats), *B. canis* (dogs), and *B. ovis* (sheep). All except *B. ovis* and *B. neotomae* are known to be infectious to humans.

Each species can be further subdivided into biovars. Some controversy exists over whether the six species should be considered serovars of a single species, due to high DNA homology among them. In addition, a number of *Brucella* strains isolated from marine mammals await further genetic classification.

The resolution of species has been dependent on host preference; outer-membrane protein sequences; small, but consistent, genetic differences; biochemical characteristics; and restriction maps. For example, slide agglutination is very useful for distinguishing "smooth" strains (i.e., those with an *O*-polysaccharide-containing outer-membrane lipopolysaccharide: *B. melitensis, B. abortus*, *B. suis*, and *B. neotomae*) from "rough" strains (i.e., those without an *O*-polysaccharide-containing outer-membrane lipopolysaccharide: *B. ovis* and *B. canis*).

Wildlife reservoirs of *B. abortus* also exist in free-roaming elk and bison.

2. Disease

Brucellosis transmitted from animal hosts to humans (i.e., zoonotic) is highly contagious, but is rarely transmitted from human to human. Contact occurs most commonly through occupational exposure (e.g., assisting with animal birthing) or ingestion of animal products (e.g., raw milk and soft cheeses made with unpasteurized goat or cow milk). Among the rare instances of human-to-human transmission are those that have included exposure through reproduction and breast-feeding.

In addition to depending on the type of *Brucella* strain, the severity of the illness depends on host factors and dose.

Vaccines are routinely used to control disease in livestock. Certain vaccine strains, notably *B. abortus* RB51, and inadvertent needle sticks have resulted in infection in humans.

Currently no vaccine exists for humans.

- **Mortality**: Less than 2%.

- **Infective dose:** Undefined for humans; however, it is estimated that fewer than 500 cells are enough to establish infection. Humans appear to be more susceptible to *B. melitensis* than to the other species that infect humans.

- **Onset**: Following exposure, signs of illness usually appear within 3 weeks, but longer incubation periods are not unusual.

- **Disease / complications:** In the beginning stage of illness, septicemia results after multiplication of the organism in regional lymph nodes. Patients have the intermittent fevers and sweating that are the hallmarks of brucellosis, along with other potential symptoms (described in Symptoms section, below).

  If the diagnosis of brucellosis is delayed or the disease is left untreated, the disease may become chronic, and focalizations of brucellosis in bones (i.e., brucellar spondylitis) and joints may occur. Other potential complications include bacterial endocarditis, meningioencephalitis, and myocarditis. Allergic hypersensitivity (dermal) is not uncommon and should be a consideration for laboratory workers or others with repeated exposures to the organism or antigens.

The antibiotics most commonly used to treat human brucellosis include tetracycline, rifampicin, and the aminoglycosides. However, due to a high likelihood of relapse, health officials recommend the administration of more than one antibiotic for up to 6 weeks. Common combinations include doxycycline plus rifampicin or doxycycline plus streptomycin. For approximately 90% of patients, such aggressive therapy is enough to treat the infection and prevent relapse.

- **Symptoms**: Potential initial signs of illness include intermittent (i.e., "undulant") fever, chills, sweating, weakness, malaise, headache, and joint and muscle pain. Patients who develop complications may show symptoms of endocarditis or myocarditis, such as shortness of breath, arrhythmia, edema, or chest pain; meningoencephalitis, such as severe headache, stiff neck, confusion, or seizures; or spondylitis, such as back pain.

- **Duration**: With appropriate antibacterial therapy, it is possible to see resolution of disease in only a few weeks; however, even with treatment, symptoms may reappear and last for months or even years.

- **Route of entry**: Oral; e.g., through ingestion of contaminated raw milk or milk products. Inhalation; e.g., by laboratory personnel in the clinical setting. Via skin wounds; e.g., in slaughterhouse workers and veterinarians. In rare instances, human-to-human transmission may occur through, e.g., reproduction or breast-feeding.

- **Pathway:** Humans most commonly come into contact with *Brucella* through cutaneous, respiratory, or gastrointestinal routes of exposure, allowing the bacteria access to both the blood and reticuloendothelial system. How *Brucella*, an intracellular parasite, survives intracellularly and its pathogenesis pathway in humans are not well understood. It is clear that the organism's ability to live and replicate within the phagocytic cells of the reticuloendothelial system (e.g., macrophages) is a critical component of its ability to evade host defenses and establish disease chronicity. Once inside the macrophage, some bacteria are killed; however, a subpopulation can be transported into the intracellular spaces (i.e., the replicative phagosome) of the macrophage and multiply unnoticed and without inducing cell death. When moved to the lymph nodes, macrophages die and can release large amounts of bacteria.

  In humans, the infection is primarily focused within the reticuloendothelial system, but, in other animal hosts, the organism targets the placental trophoblast cells of pregnant animals, causing fetuses to be aborted. Human cases of spontaneous abortion have been noted following infections with Brucella, similar to occurrences associated with another intracellular pathogen, *Listeria monocytogenes*, that likewise affects dairy products.

  Research on *Brucella* pathogenesis has revealed one reason *B. melitensis* might be more pathogenic to humans than are other species. Study of human neutrophils found in the bloodstream demonstrated different responses for different species of *Brucella*. For example, the bacteria were killed more readily in neutrophils infected with *B. abortus* than in those infected with *B. melitensis*. However, strains of *B. abortus* and*B. melitensis* in which the virulence was attenuated showed no difference.

Effects of *Brucella* on animal hosts: *Brucella* species generally do not cause illness in their primary (animal) hosts. In many cases, the only evidence of infection appears when a pregnant host suffers an abortion. Male animals can asymptomatically harbor the organism in their reproductive organs. Although *Brucella* strains have a strong preference for their host animals,

interspecies transmission does occur through close physical contact with the bacterium. *B. ovis* and *B. canis* appear to have a substantially reduced virulence for animals other than their hosts.

3. Frequency

According to a recent estimate by the Centers for Disease Control and Prevention (CDC), 839 cases of foodborne brucellosis occur each year in the United States, if under-reporting and under-diagnosis are taken into account. Vaccination of domestic livestock has largely controlled the disease in the U.S. and Canada.

4. Sources

Brucellosis in humans is usually associated with consumption of unpasteurized milk and soft cheeses made from the milk of infected animals.

5. Diagnosis

Most often, the diagnosis of brucellosis relies on the isolation of the organism from blood or bone marrow. In addition, a number of immunologic techniques exist for detection of anti-*Brucella* antibodies. The organism may also be isolated from the liver, spleen, bone marrow, or cerebrospinal fluid.

The growth of *Brucella* from blood culture is notoriously slow, contributing to difficulties in diagnosis.

In the case of disease progression to focalizations or chronic infections, histologic changes and radiologic evidence of erosion of lumbar vertebrae are useful for diagnosis. Localization of infection in the spinal column, brucellar spondylitis, is not uncommon with chronic infection. Appearance of Pedro Pons' sign (erosion at the anterior superior angle of lumbar vertebra) and bone spurs (osteophytosis) are classic indications of brucellar spondylitis.

6. Target Populations

Veterinarians and farm workers are at particular risk of infection, due to occupational exposure to tissues of aborted animal fetuses, which may contain millions of organisms.

Brucellosis in humans tracks the distribution of animal illness. Human cases of brucellosis are found primarily in developing countries with animal cases and a high level of consumption of unpasteurized milk products.

In the U.S., human cases linked to domestically produced milk or milk products are largely nonexistent; cases are almost exclusively linked to unpasteurized milk products imported from certain areas of Latin America. This is in contrast to countries such as Mexico, where both human and animal infection of *B. melitensis* have been reported in every state.

Other focal points for both animal and human infection caused by *B. melitensis* include countries with large goat populations, including Mediterranean Europe, Africa, the Middle East, India, and parts of Asia.

► ***Brucellosis is the most commonly reported laboratory-acquired infection among clinical laboratory personnel.*** ◄ Risk of transmission arises during laboratory procedures that cause the

organism to become airborne (e.g., pouring of broths, sample centrifugation). For this reason, all manipulations generating bioaerosols should be done in a class II biological safety cabinet, using Biosafety 3 containment practices and facilities.

7. Food Analysis

Currently no method is available for routine analysis of foods for *Brucella* spp.

8. Examples of Outbreaks

CDC/MMWR Brucella – provides a link to CDC Morbidity and Mortality Weekly Reports related to *Brucella*.

MMWeeklyReport article emphasizes the occupational risk from exposure to *Brucella.*

9. Other Resources

- NIH/PubMed, *Brucella search* – Provides a list of research abstracts from the National Library of Medicine's MEDLINE database.
- Agricola, *Brucella* search – Provides a list of research abstracts from the National Agricultural Library database.
- Loci index for genome Taxonomy Browser – Available from the GenBank Taxonomy database, which contains the names of all organisms represented in the genetic databases with at least one nucleotide or protein sequence.
- CDC information about brucellosis.

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Vibrio cholerae Serogroups O1 and O139

1. Organism

Vibrio cholerae serogroups O1 and O139 are responsible for epidemics and pandemic cholera outbreaks. These organisms are Gram-negative, slightly curved, rod-shaped bacteria that occur naturally in aquatic environments. Virulence of *V. cholerae* serogroups O1 and O139 is predicted by the production of an enterotoxin called cholerae toxin (CT) and the toxin co-regulated pilus (TCP).

(Note: these organisms should not be confused with other *Vibrio* species addressed in other chapters of this book; i.e., *Vibrio cholerae* non-O1 non-O139, *Vibrio parahaemolyticus,* and *Vibrio vulnificus*.)

V. cholerae O1 and O139 are the most hardy of the pathogenic *Vibrio* spp. and have the ability to survive in freshwater and in water composed of up to ~3% salt. However, these organisms are very susceptible to disinfectants, cold temperatures (especially freezing), and acidic environments. They are readily inactivated at temperatures >45°C, and cooking food is lethal to *V. cholerae* O1 and O139. *V. cholerae* O139 is unique among *V. cholerae* strains, in that it is encapsulated. However, this does not appear to provide greater pathogenicity or resistance to common disinfectants, such as ethanol and bleach.

2. Disease

V. cholerae causes cholera, a gastrointestinal illness.

For Consumers: A Snapshot

There are different kinds (species) of *Vibrio*, a bacterium. This one causes cholera, a disease that can be mild, but sometimes becomes serious. If serious cases aren't treated, they often are fatal. This kind of *Vibrio* can live in both saltwater (for example, coastal ocean water) and freshwater, such as rivers. It grows there naturally or can get into the water from the bowel waste of infected people (sewage). Water contaminated with *Vibrio* can cause illness if people drink the water or eat seafood that has been living in it, or if the water comes into contact with food in other ways. In the U.S., occasional cases of cholera from seafood (and even small outbreaks) continue, but the problem is much larger in countries with poor sanitation. After the 2010 earthquake in Haiti, when many people had only unsanitary water for bathing and drinking, a large cholera outbreak killed more than 7,000 people. This bacterium makes a toxic substance, in the bowel, that causes watery diarrhea. Vomiting also may occur. Symptoms start a few hours to 3 days after contaminated food or water is taken in. In mild cases, the illness usually goes away by itself in a few days. In serious cases, there may be so much diarrhea that the body loses dangerous amounts of fluid and minerals – so much that, without treatment, patients may die. Health professionals can provide the right balance of fluid and minerals and, if needed, the right kinds of antibiotics to kill *Vibrio*. But preventing the illness in the first place is a better idea. You can help protect yourself by cooking seafood until the inside reaches a temperature, for at least 15 seconds, of 145°F, but 155°F for things like fishcakes and 165°F for stuffed fish. It's important to wash raw foods in sanitary water and to wash hands, equipment, and cooking and food-handling surfaces, and to keep food refrigerated at 40°F or lower. After kitchen surfaces are washed, sanitize them with a commercially available product that's sold as a kitchen sanitizer. Cooked foods should always be kept from touching raw foods, to prevent contamination. That's especially important with this kind of *Vibrio*, which can grow in cooked food if it becomes contaminated. You might have heard people say that you should eat oysters or other shellfish only in months with the letter "R" – for example Janua**r**y, Feb**r**ua**r**y, etc. But remember that *Vibrio* and other bacteria (and viruses) that affect seafood can cause illness in *any* month, so follow basic food-safety tips all year.

- **Mortality:** Without rehydration therapy, this disease has a 30% to 50% mortality rate; however, with timely treatment, the fatality rate is less than 1%.

- **Infective dose:** It is estimated that ingestion of 1 million organisms is required to cause illness.

- **Onset:** Symptoms usually appear within a few hours to 3 days of ingestion.

- **Illness / complications:** Infection with *V. cholerae* serogroups O1 or O139 causes mild to severe diarrhea. Approximately 20% of those infected have watery diarrhea, and 10% to 20% of those develop severe watery diarrhea (characteristic rice-water stools) and vomiting.

  Cholera gravis, the most severe form of cholera infection, is characterized by severe fluid and electrolyte loss from vomiting and profuse, watery diarrhea. Complications include tachycardia, hypotension, and dehydration.

  V. cholerae O1 and O139 infections can be treated with antibiotics, though rehydration therapy is generally sufficient. Doxycycline and/or tetracycline are the antibiotics of choice; however, some resistance to tetracycline has been reported.

- **Symptoms:** The illness generally presents with abdominal discomfort and diarrhea that may vary from mild and watery to acute, with rice-water stools. Vomiting also occurs in some cases.

- **Duration**: Mild gastroenteritis cases usually resolve within a few days of symptom onset. Cases requiring medical intervention via rehydration therapy or antibiotic treatment can persist longer, depending on severity of illness when treatment is initiated.

- **Route of entry**: Oral.

- **Pathway**: CT is an enterotoxin that enters epithelial cells of the intestine and causes secretion of electrolytes and water into the lumen of the intestine. This water loss results in severe diarrhea and dehydration. It is known that CT is a multi-subunit toxin encoded by the *ctx*AB operon. Additionally, genes responsible for formation of the TCP (toxin co-regulated pilus) are essential for infection.

3. Frequency

No major outbreaks of cholera have occurred in the United States since 1911. However, sporadic cases and small outbreaks have been reported since 1973, suggesting an environmental reservoir in the U.S. The Centers for Disease Control and Prevention (CDC) estimates that 84 cases of foodborne cholera occur in the U.S. annually. This organism causes an estimated 11 million cases per year worldwide, excluding outbreaks. Nearly 90% of cases and 70% of outbreaks from 1995 to 2005 occurred in Africa.

4. Sources

In the U.S., infections with these organisms have been associated with a variety of seafoods, including molluscan shellfish (oysters, mussels, and clams), crab, lobster, shrimp, squid, and finfish. Illness generally results from consumption of these seafoods raw, improperly cooked, or

cross contaminated by a raw product. Although cooking kills these bacteria, serogroups O1 and O139 can grow in shellfish that have been contaminated *after* cooking, and prompt refrigeration of food remnants is important for prevention of this illness.

In areas where *V. cholerae* serogroup O1 and/or O139 is endemic, infections can occur from ingestion of water; ice; unwashed, contaminated food; and seafood.

5. Diagnosis

Cholera can be confirmed only by isolation of the causative organism from the diarrheic stools of infected people.

6. Target Populations

All people are believed to be susceptible to infection. However, infection is more likely to occur among people in impoverished areas, poorly developed areas, and areas with a high population density. Cholera is most severe in children suffering from malnutrition. People who have not previously been exposed to the organism are more likely to become infected, as immunity is usually conferred by infection. Improved sanitation and hygiene can help prevent the disease.

7. Food Analysis

FDA's Bacteriological Analytical Manual (BAM) describes the methods most commonly used to isolate this organism from foods. Pathogenic and non-pathogenic forms of the organisms do exist; therefore, testing food isolates for the virulence determinants is recommended. The BAM recommends a PCR method for detection of the gene responsible for cholera toxin (CT) production.

8. Examples of Outbreaks

In the U.S., two cases of cholera were reported following Hurricanes Katrina and Rita, in 2005.

Internationally, the reported outbreak of cholera that occurred in Haiti, in October 2010, included an estimated 530,000 illnesses and at least 7,000 deaths.

As is the case with the other *Vibrio* spp., there is a seasonal trend associated with outbreaks; illnesses are more likely to occur in the warmer months.

9. Other Resources

Centers for Disease Control and Prevention disease listing. General information about *V. cholerae*.

World Health Organization. General and technical information about cholera, including outbreak details.

Risk assessment of choleragenic *Vibrio cholerae* O1 and O139 in warm-water shrimp in international trade. This risk assessment structures knowledge about *V. cholerae* O1 and O139 in a systematic manner, and includes mathematical models developed to estimate exposure to this microorganism and the dose-response relationships.

National Center for Biotechnology Information Taxonomy. Provides information about the historical classification of *V. cholerae* as well as current genetic sequence information.

Additional reading:

FAO/WHO. 2005. Risk Assessment of Choleragenic *Vibrio cholerae* O1 and O139 in Warm-Water Shrimp in International Trade: Interpretative Summary and Technical Report in World Health Organization, Food and Agriculture Organization of the United Nations, Geneva, Switzerland.

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Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Vibrio cholerae non-O1 non-O139

1. Organism

This Gram-negative, curve-shaped bacterium is naturally occurring in brackish (i.e., somewhat salty) water, but survives and occurs in aquatic environments ranging from freshwater to open ocean. Non-O1 non-O139 *Vibrio cholerae* typically do not produce cholera toxin (CT), and little is known about how these organisms cause disease. The only serogroups of *V. cholerae* currently recognized as causing cholera are O1 and O139; however, cholera-like symptoms have been infrequently reported in the United States from CT-producing strains from serogroups O141 and O75.

(Note: This organism should not be confused with other *Vibrio* serogroups or species addressed in other chapters of this book; i.e., *Vibrio cholerae* O1 and O139, which does cause cholera; *Vibrio parahaemolyticus*; and *Vibrio vulnificus*.)

V. cholerae non-O1 non-O139 are more hardy than most of the other pathogenic *Vibrio* spp. and have the ability to survive in freshwater and in water composed of up to ~3% salt. However, these organisms are very susceptible to cold temperatures, including freezing, and acid environments. Additionally, cooking food thoroughly kills *V. cholerae* non-O1 non-O139. *V. cholerae* non-O1 non-O139 are not encapsulated, and are susceptible to common disinfectants, such as ethanol and bleach.

For Consumers: A Snapshot

There are different kinds (species) of *Vibrio*, a bacterium. This one doesn't cause cholera (that type of *Vibrio* is covered in another chapter), but can cause diarrhea, stomach cramps, fever, nausea, and/or vomiting, which usually go away by themselves in about a week. In people with weak immune systems, it can go on to infect the blood and cause serious or deadly infections in other parts of the body, and about 5% of those people die each year. Examples of people with weak immune systems are those with HIV/AIDS or who are on medicines that lower the actions of the immune system, like some kinds of drugs for rheumatoid arthritis or cancer treatment. These people, especially, should always thoroughly cook their seafood, and should see a health professional if they develop symptoms. This kind of *Vibrio* usually lives in water that's mildly salty, but also can live in the ocean and fresh inland waters, such as rivers. It can also get into the water from the bowel waste of infected people (for example, from sewage). Water contaminated with *Vibrio* can cause illness if people drink the water or eat seafood that has been living in it, or if the contaminated water comes into contact with food in other ways. In the U.S., more than 17,000 cases of this illness occur each year. You can help protect yourself by cooking seafood until the inside reaches a temperature, for at least 15 seconds, of 145°F, but 155°F for things like fishcakes and 165°F for stuffed fish. It's important to wash raw foods in sanitary water and to wash hands, equipment, and surfaces when handling or cooking food; keep food refrigerated at 40°F or lower; and keep raw foods from touching cooked foods and equipment and surfaces used for cooking or eating. After kitchen surfaces are washed, sanitize them with a commercially available product that's sold as a kitchen sanitizer. You might have heard people say that you should eat oysters or other shellfish only in months with the letter "R" – for example Janua**r**y, Feb**r**ua**r**y, etc. But remember that *Vibrio* and other bacteria (and viruses) that affect seafood can cause illness in *any* month, so follow basic food-safety tips all year long.

2. Disease

Non-Ol non-O139 *V. cholerae* causes gastroenteritis, but not cholera. Occasionally, it causes septicemic infections among people with predisposing conditions. Those conditions include chronic liver disease (cirrhosis, hepatitis, liver transplantation, and cancer of the liver), elevated serum iron levels (hemachromatosis), compromised immune system (for example, chemotherapy, steroid use and other immunosuppressive medications, AIDS), other chronic illnesses (diabetes, renal disease, intestinal disease, and insufficient gastric acid). People with these conditions, especially, should eat seafood only if it has been properly cooked.

- **Mortality**: The fatality rate is about 5%, generally among people with the predisposing conditions listed above.
- **Infective dose:** It is suspected that large numbers (more than 1 million) of the organism must be ingested to cause illness.
- **Onset**: Symptoms usually appear within 1 to 3 days of ingestion.
- **Illness / complications:** Diarrhea resulting from ingestion of this organism is generally self-limiting. However, septicemia infections can result, and there is approximately a 5% fatality rate associated with non-O1 non-O139 *V. cholerae*, generally in people having predisposing conditions similar to those for *V. vulnificus* infection.
- **Symptoms**: Diarrhea, abdominal cramps, and fever are the predominant symptoms associated with this illness, with vomiting and nausea occurring in approximately 25% of infected people. Approximately 25% of infected people have blood and mucus in their stool.
- **Duration**: Symptoms usually resolve within 7 days.
- **Route of entry**: Oral. (Occasionally, infections with this organism that are not foodborne occur in wounds and ears.)
- **Pathway**: Very little is known about how non-CT producing strains of *V. cholerae* cause disease. These strains generally produce other types of enterotoxins, such as RTX (repeats in toxin); however, none have been shown to be absolutely necessary for infection.

3. Frequency

The Centers for Disease Control and Prevention (CDC) estimates that 17,564 cases of foodborne illness from these *Vibrio* species occur annually in the U.S. In the spring of 2011, the first oyster-associated *V. cholerae* O75 outbreak in the U.S. occurred. There were 10 illnesses associated with consumption of raw oysters from Florida. (See Onifade TJM, Hutchison R, Van Zile K, Bodager D, Baker R, Blackmore C. 2011. Toxin producing *Vibrio cholerae* O75 outbreak. United States, March to April 2011. Eurosurveillance. 16(20):pii=19870.

4. Sources

Sporadic cases generally occur along the coasts of the U.S. and are associated with consumption of raw, improperly cooked, or cross-contaminated seafood during the warmer months.

5. Diagnosis

Diagnosis of a *V. cholerae* infection is made by culturing the organism from patients' diarrheic stool or from the blood of patients with septicemia.

6. Target Populations

Anyone who eats raw shellfish is susceptible to diarrhea caused by this organism. As noted above, cirrhotic or immunocompromised people may develop severe complications, such as septicemia.

7. Food Analysis

FDA's Bacteriological Analytical Manual (BAM) describes the methods most commonly used to isolate this organism from foods. Pathogenic and non-pathogenic forms of the organisms do exist; therefore, testing food isolates for the virulence determinants is recommended. The BAM recommends a PCR method for the detection of the gene responsible for CT production.

8. Examples of Outbreaks

This organism generally is associated with sporadic illnesses and rarely causes outbreaks. In the spring of 2011, the first oyster-associated *V. cholerae* O75 outbreak in the U.S. occurred. There were 10 illnesses associated with consumption of raw oysters from Florida. (See Onifade TJM, Hutchison R, Van Zile K, Bodager D, Baker R, Blackmore C. 2011. Toxin producing *Vibrio cholerae* O75 outbreak, United States, March to April 2011. Eurosurveillance. 16(20):pii=19870.)

9. Resources

CDC disease listing – General information about *V. cholerae*.

National Center for Biotechnology Information Taxonomy. Information about the historical classification of *V. cholerae*, as well as current genetic sequence information.

Additional reading:

FAO/WHO, 2005. Risk Assessment of Choleragenic *Vibrio cholerae* O1 and O139 in Warm-Water Shrimp in International Trade: Interpretative Summary and Technical Report, World Health Organization / Food and Agriculture Organization of the United Nations, Geneva, Switzerland.

CDC. 2008. Summary of human *Vibrio* cases reported to CDC, 2007.

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Vezzulli L, Guzman CA, Colwell RR, Pruzzo C. 2008. Dual role colonization factors connecting *Vibrio cholerae*'s lifestyles in human and aquatic environments open new perspectives for combating infectious diseases. Curr. Opin. Biotechnol. 19:254-259.

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Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Vibrio vulnificus

1. Organism

This Gram-negative, curve-shaped bacterium is found in estuarine environments and is associated with various marine species, such as plankton, shellfish, crustaceans, and finfish. It is found throughout coastal waters of the continental United States.

Optimal temperatures for *V. vulnificus* are between 20°C to 35°C; it can grow at temperatures up to 41°C. It is slowly inactivated at temperatures <10°C (minimum growth temperature), and cultures should never be stored in refrigerators. *V. vulnificus* is halophilic; the highest abundance in oysters is at 23ppt. It is lysed almost immediately in freshwater; thus, it is not usually transmitted via the fecal-oral route. At least 0.5% NaCl is required in all media, and 2% NaCl is optimal. Like other vibrios, *V. vulnificus* is highly susceptible to low pH, freezing, and cooking. Most strains of *V. vulnificus* produce a capsule, but all strains can be killed by common disinfectants, such as bleach and alcohol.

2. Disease

Although illness from this *Vibrio* species is less common than that from other *Vibrio* species (which are addressed separately, in other chapters), it more often tends to be deadly. If the infection is detected early, *V. vulnificus* is susceptible to treatment with antibiotics; generally tetracycline.

For Consumers: A Snapshot

There are different kinds (species) of *Vibrio*, a bacterium. This one doesn't cause cholera (that type of *Vibrio* is covered in another chapter), and it doesn't cause illness as often as the other kinds – just under 100 cases a year – but when it does, the illness is more often fatal. If it's detected early, certain antibiotics can be used to treat it. This kind of *Vibrio* usually lives in estuaries; for example, where sea water and river water come together. Water contaminated with *Vibrio* can cause illness if people drink the water or eat seafood (shellfish, such as oysters and clams, and shrimp, as a few examples) that has been living in it, or if the contaminated water comes into contact with food in other ways. Cooked foods should always be kept from touching raw foods, to prevent contamination. That's especially important with this kind of *Vibrio*, which grows easily in cooked food if it becomes contaminated. In people with weak immune systems, especially, illness from this kind of *Vibrio* can go on to infect the blood and cause serious or deadly infections in other parts of the body, too. About 35% of people in whom the infection has spread to the blood die. A few examples of people with weak immune systems are those with HIV/AIDS or who are on medicines that lower the actions of the immune system, like some drugs for rheumatoid arthritis or cancer treatment. People with high levels of iron in their blood, usually due to liver disease, also are at higher risk. These people, especially, who are at higher risk for whatever reason, should always thoroughly cook their seafood and should see a health professional if they develop symptoms. You can help protect yourself by cooking seafood until the inside reaches a temperature, for at least 15 seconds, of 145°F, but 155°F for fishcakes and 165°F for stuffed fish. It's also important to wash raw foods in sanitary water and to wash hands, equipment, and surfaces when handling or cooking food; keep food refrigerated at 40°F or lower; and keep raw foods from touching cooked foods and equipment and surfaces used for cooking or eating. After kitchen surfaces are washed, sanitize them with a commercially available product that's sold as a kitchen sanitizer. You might have heard people say that you should eat oysters or other shellfish only in months with the letter "R" – for example Janu**a**r**y**, Feb**r**u**a**r**y**, etc. But remember that *Vibrio* and other bacteria (and viruses) that affect seafood can cause illness in *any* month, so follow basic food-safety tips all year.

- **Mortality:** Death occurs in an average of 35% of septicemia cases (and 20% of wound-infection cases).

- **Infective dose:** The infective dose from ingestion of *V. vulnificus* is largely unknown, since human feeding studies involving this organism would be unethical. The FAO/WHO *V. vulnificus* Risk Assessment (VVRA) provides a dose response based on U.S. epidemiologic data and estimates that (1) a dose of 1,000 organisms can cause illness and (2) at a total dose of 1 million organisms, the risk of disease for susceptible people is 1:50,000.

- **Onset:** The range of time to onset of gastroenteritis symptoms may be approximately 12 hours to 21 days. (Onset of symptoms in cases of wound infection may be in as few as 4 hours.) The mean time to septicemia is 4 days.

- **Illness / complications**: In healthy people, ingestion of this organism can cause gastroenteritis that generally remains localized and is self-limiting. Among susceptible people, the organism may cause primary septicemia (septic shock). Susceptible people include those with a predisposing condition; for example, those who are immunocompromised or have high serum iron levels (usually due to liver disease). More than 60% of those with septicemia develop secondary lesions on the extremities, similar to those found in wound infections.

  V. vulnificus also can cause wound infections directly, either through wounds incurred while handling fish, crustaceans, or shellfish, or when a pre-existing wound is exposed to marine or estuarine waters harboring the organism. Wound infections caused by *V. vulnificus* are characterized by inflammation at the wound site, which can progress to cellulitis, bullous lesions, and necrosis. The infection can become systemic, with affected people developing fever, chills, altered mental status, and hypotension.

  Secondary lesions from septicemia, as well as primary wound infections caused by direct contact, frequently require surgical debridement or amputation.

- **Symptoms:** Gastroenteritis caused by *V. vulnificus* is characterized by fever, diarrhea, abdominal cramps, nausea, and vomiting. Onset of septicemia is characterized by fever and chills, occasionally accompanied by vomiting, diarrhea, abdominal pain, and/or pain in the extremities.

- **Duration:** In uncomplicated cases, gastroenteritis is self-limiting. The mean duration of septic illness is 1.6 days (i.e., the brief duration is reflective, in part, of the high mortality associated with septicemia).

- **Route of entry:** The gastroenteritis form of illness caused by *V. vulnificus* results from ingestion of the organism.

- **Pathway:** *V. vulnificus* harbors many putative virulence factors, including capsule, pili, hemolysins, metalloproteases, and enterotoxins. However, none of these factors has been shown unequivocally to be essential in causing disease; much remains unknown.

3. Frequency

Sporadic illnesses have been attributed to this organism, but no foodborne outbreaks have been reported. The Centers for Disease Control and Prevention (CDC) estimates that 96 cases of foodborne illness from *V. vulnificus* occur annually in the U.S. Sporadic cases are more prevalent during the warmer months, when water temperatures are higher than 20°C (68°F).

4. Sources

More than 90% of *V. vulnificus* illnesses in the U.S. are associated with consumption of raw Gulf Coast oysters. Ingestion of clams and shrimp also has been associated with disease. Thorough cooking or freezing kills the organism, so illnesses usually occur from consumption of raw seafood or cooked seafood that has been contaminated with raw product.

5. Diagnosis

The culturing of the organism from wounds, diarrheic stools, or blood is diagnostic of this illness.

6. Target Populations

Anyone who eats raw seafood products harboring *V. vulnificus*, or cooked seafood products cross contaminated with the organism, may develop gastroenteritis. People with predisposing conditions are the most susceptible to septicemia and should eat seafood products only if they have been properly cooked. Predisposing conditions include chronic liver disease (cirrhosis, hepatitis, liver transplantation, or cancer of the liver), elevated serum iron levels (hemachromatosis), compromised immune system (chemotherapy, steroid or other immunosuppressive medication use, AIDS), other chronic illnesses (diabetes, renal disease, intestinal disease), and insufficient gastric acid.

Anyone may develop wound infections from contact with estuarine waters.

7. Food Analysis

FDA's Bacteriological Analytical Manual (BAM) describes the methods most commonly used to isolate this organism from foods. More recent molecular methods are available that can be applied directly to seafood products to screen for the presence of *V. vulnificus* prior to isolation.

8. Examples of Outbreaks

No outbreaks of *V. vulnificus* have been reported. Sporadic cases occur throughout the year, increasing in frequency during the warmer months.

Additional illness information can be found in CDC's Morbidity and Mortality Weekly Reports.

9. Resources

National Center for Biotechnology Information Taxonomy provides information on the historical classification of *V. vulnificus*, as well as current genetic sequence information.

The CDC Disease Listing provides information about *V. vulnificus.*

FAO/WHO Risk Assessment of *Vibrio vulnificus* in Raw Oysters structures knowledge about *V. vulnificus* in a systematic manner and includes mathematical models developed to estimate exposure to this microorganism.

Interstate Shellfish Sanitation Conference is a cooperation of state and federal control agencies, the shellfish industry, and the academic community that promotes shellfish sanitation and provides educational material.

Safe Oysters. A gateway to *Vibrio vulnificus* information for health care providers, food and health educators, consumers, fishermen, and commercial processors.

***Additional Reading*:**

Food and Agricultural Organization and World Health Organization, 2010. Risk Assessment of *Vibrio vulnificus* in Raw Oysters: Interpretative Summary and Technical Report, World Health Organization / Food and Agriculture Organization of the United Nations, Rome, Italy.

Haq SM, Dayal HH. 2005. Chronic liver disease and consumption of raw oysters: a potentially lethal combination--a review of *Vibrio vulnificus* septicemia. Am. J. Gastroenterol. 100:1195-1199.

Strom MS, Paranjpye RN. 2000. Epidemiology and pathogenesis of *Vibrio vulnificus*. Microbes. Infect. 2:177-188.

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Foodborne Pathogenic Microorganisms and Natural Toxins

Cronobacter species

(formerly *Enterobacter sakazakii)*

1. Organism

Cronobacter, formerly *Enterobacter sakazakii*, is a Gram-negative, motile, rod-shaped, non-sporulating pathogenic bacterium that can cause foodborne illness, primarily among infants and immunocompromised adults. It is a rare cause of invasive diseases, including bacteremia, meningitis, and necrotizing enterocolitis.

The organism is able to survive in low-moisture foods, such as powdered infant formula, for long periods.

■ Notes on nomenclature change from *E. sakazakii*:

Cronobacter originally was defined as a species, *Enterobacter sakazakii*, in 1980. New evidence obtained through recent research using amplified fragment-length polymorphism, phenotypic arrays, automated ribotyping, 16S rRNA gene sequencing, and DNA-DNA hybridization resulted in a nomenclature change, in 2008, from *E. sakazakii* to a new genus, *Cronobacter*.

Five species comprise the new genus, including *Cronobacter sakazakii* gen. nov., *Cronobacter malonaticus* sp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., and *Cronobacter dublinensis* sp. nov. An additional new species, *Cronobacter* genomospecies 1, also has been proposed. However, a very limited number of type strains have been identified for this species.

For Consumers: A Snapshot

This bacterium is especially risky for newborn infants. The illness it causes is rare, but when it occurs, infants younger than 2 months old are at highest risk. The death rate is high, from 10 percent to 80 percent. Unlike some bacteria, this one can survive in dried foods, like powdered infant formula. It can then multiply after liquid is added to the formula, especially if the formula is stored at an incorrect temperature, and cause illness in babies who drink it. Symptoms may include poor feeding, irritability, jaundice (yellow skin and whites of the eyes, which can also be caused by other conditions), temperature changes, grunting breaths, and seizures. The infection may cause bowel damage and may spread through the blood to other parts of the body, such as the brain, causing permanent damage in those who survive. Although the bacterium also has been found in a variety of other foods, only powdered infant formula has been linked to cases of illness, and this bacterium doesn't usually cause illness in otherwise healthy people. Following basic food-safety tips can help prevent infections, and the information about infant-formula, below, also provides important tips.

Follow the directions on infant-formula labels carefully. Use liquid heated to 158°F to 194°F. (Don't use a microwave; the top of the liquid might feel warm, but other parts might be too hot). With the formula in the bottle, run the lower part of the bottle under cold water or put it in an ice bath to cool it quickly, so it doesn't have to sit at room temperature. Dry off the wet part, but not the top (which should not have gotten wet). Another choice: the liquid formula in stores is required to be sold to you already sterilized by the manufacturer. After infant formula has been prepared, it can be stored in the refrigerator, at 40°F or below, for up to 24 hours, but not more than 2 hours at room temperature. Take formula out of the refrigerator and rewarm it only if you plan to use it immediately. Don't rewarm it for more than 15 minutes, and throw away any that isn't used within 2 hours. All through this process, use good hygiene, like handwashing. It's also important to clean and sanitize sinks and counter tops where formula is prepared. Reusable bottles and bottle nipples should be cleaned with soap and water and can be sanitized by boiling in water for a few minutes.

Three subspecies of *dublinensis* sp. nov. have been proposed, which include *Cronobacter dublinensis* subsp. *dublinensis* subsp. nov., *Cronobacter dublinensis* subsp. *lausannensis* subsp. nov., and *Cronobacter dublinensis* subsp. *lactaridi* subsp. nov.

2. Disease

- **Mortality**: The infection usually has a very high case-fatality rate, which ranges from 10% to 80%. Newborn infants are particularly at risk; infants older than 6 months rarely are affected. Higher case-fatality rates are often associated with premature or low-birthweight infants. In recent years, the highest mortality has been in healthy term infants who developed septicemia.

- **Infective dose:** The infective dose has not been determined, but scientists have speculated that a reasonable estimate might be similar to that of *Escherichia coli* O157:H7 (that is, low; e.g., 10 to 100 organisms).

- **Onset**: Symptoms occur in infants within a few days. The onset in adults is unknown, as cases in adults have been rare and the food sources usually have not been determined.

- **Illness / complications:** *Cronobacter* can cause bloodstream and central nervous system infections. The organism also has been associated with sepsis, meningitis, and necrotizing enterocolitis, although it has not been firmly established as a causative agent. Meningitis survivors may develop severe neurologic complications.

- **Symptoms**: Symptoms are often severe and may include poor feeding response, irritability, jaundice, grunting respirations, instability of body temperature, seizures, brain abscess, hydrocephalus, and developmental delay.

- **Duration of symptoms**: Among survivors, colonization varies from 2 to 8 weeks. Among fatalities, death may occur within a few hours to several days after the first signs of sepsis appear.

- **Route of entry**: Most infections are caused by oral entry, although rare cases of wound infection also have been reported.

- **Pathway**: The pathogenesis of *Cronobacter*-induced neonatal meningitis and enterocolitis is not fully understood. The organism appears to adhere to host cell surfaces instantaneously, then reproduce to an optimal concentration. The adhesion of *Cronobacter* to epithelial cells is mainly non-fimbriae-based, and other, unidentified virulence factors also might contribute to the binding**.**

3. Frequency

Relatively few cases of *Cronobacter* infection have been documented, and the organism has rarely been isolated from food products and clinical samples. Since 1958, there have been 120 reported cases of *Cronobacter* infection in infants, with an average of fewer than 5 reported cases, per year, worldwide. Some epidemiologic studies suggest a *Cronobacter* infection rate of less than 1% among patients with suspected symptoms. However, this does not take into account potential false-negative identifications.

4. Sources

Cronobacter infections in infants often have been associated with contaminated powdered infant formula products. *Cronobacter* has been isolated from powdered infant formula, rehydrated infant formula, and utensils used to prepare infant formula.

Powdered infant formula is not sterile, and its nutrients provide good conditions for the growth of *Cronobacter* after reconstitution. It has an a_w of ca. 0.2. *Cronobacter* is able to survive such dry conditions. Survival of *Cronobacter* in powdered infant formula for up to 2 years has been reported. The capsule formation of *Cronobacter* may contribute to its strong desiccation resistance. Because *Cronobacter* does not survive pasteurization used in powdered milk production, it has been suggested that *Cronobacter* contamination happens mainly following the spraying dry step. This could be due to either a contaminated post-drying environment or addition of ingredients that are heat-sensitive, but are added after pasteurization treatment.

Some early surveys reported a 10% to 15% contamination rate of *Cronobacter* in infant formula products, with less than 1 CFU/ 25 g in all samples. Some recent surveys isolated *Cronobacter* from 2% to 10% infant formulas, dried infant foods, milk powders, cheese products, and other dried foods.

Foods other than infant formula rarely have been associated with *Cronobacter*. However, it has been isolated from bread, cereal, rice, fruit, vegetables, legume products, herbs, spices, milk, cheese, meat, and fish. It has also been isolated from the environment of these foods' processing facilities.

5. Diagnosis

Identification of culture isolated from tissue, blood, cerebrospinal fluid, or urine aspirated through the bladder wall is necessary for diagnosis of *Cronobacter*-associated diseases.

6. Target Populations

Cronobacter infections often are associated with newborns and infants. Neonatal infections may result from contact with *Cronobacter* in the birth canal or through post-birth environmental sources. Immunosuppression, premature birth, and low birth weight may increase the risk of infection.

Approximately 50% of the children infected with *Cronobacter* are younger than 1 week old, and 75% of the children infected are younger than 1 month old. Adults are considered a low-risk group; however, a few cases of *Cronobacter* infections in immunocompromised and elderly adults also have been reported.

7. Food Analysis

In 2002, FDA devised a method for the detection of *Cronobacter* in powdered infant formula, which involved enrichment in water and *Enterobacteriaceae* enrichment broth, and plating on violet red bile glucose agar and Trypticase Soy Agar. However, this method is time-consuming, and evidence showed that it offered poor selectivity for *Cronobacter* in the presence of competing background flora. Recently, FDA completed the validation of a new method for the

detection of *Cronobacter* in powdered infant formula, which uses real-time PCR technology to significantly improve the performance of the detection.

8. Examples of Outbreaks

Memphis, Tennessee, 1988 – An outbreak of *Cronobacter*-induced septicemia and meningitis was associated with powdered infant formula contaminated with *Cronobacter*. Four neonates were involved in the outbreak, and a blender was suggested as the possible contamination source. All isolates from the infant formula and the infants had the same plasmid profile and multilocus enzyme profile.

Knoxville, Tennessee, 2001 – An outbreak of *Cronobacter* infections was linked to a powdered infant formula specific for individuals with special nutritional needs and malabsorption problems. Ten infants tested positive for *Cronobacter*, and one died following antibiotic treatment.

New Mexico, 2009 – An outbreak of *Cronobacter* infections involved two unrelated infants. No other common exposures other than infant formula were identified for the two infants, who were fed the same brand. However, *Cronobacter* culture isolated from clinical samples demonstrated different pulsed-field gel electrophoresis (PFGE) patterns. A sample from an opened can of powdered infant formula tested positive for *Cronobacter*, with a PFGE pattern that was indistinguishable from the clinical isolate from one infant.

9. Resources

- NCBI taxonomy database
- World Health Organization report from meeting on *C. sakazakii* and *Salmonella* in infant formula (Microbiological Risk Assessment Series 10).

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Aeromonas species

Aeromonas hydrophila*, *Aeromonas caviae*, *Aeromonas sobria*, *Aeromonas veronii

1. Organism

Aeromonas hydrophila is a Gram-negative, facultative anaerobic, rod-shaped bacterium that belongs to the genus *Aeromonas* and is ubiquitous in all freshwater environments and in brackish water. Some strains of *A. hydrophila* are capable of causing gastroenteritis and other infections in humans. Aeromonads also can cause illness in fish, amphibians, and domestic animals, and are listed on the Contaminant Candidate List by the Environmental Protection Agency (EPA). Some *Aeromonas* species can cause human infections, particularly in immunocompromised people.

Other *Aeromonas* infections in humans are attributed to *A. caviae* and *A. veronii* biovar *sobria*, *A. jandaei*, *A. veronii* biovar *veronii*, *A. schubertii*, and *A. trota.* It is believed that disease-causing strains are only a fraction of the diversity of strains present in the environment. However, it is difficult to identify disease-causing strains of *Aeromonas* at this time, because of a paucity of scientific information about *Aeromonas* virulence genes and associated pathogenic mechanisms (although some molecular-based methods do selectively target certain virulence genes).

The entire genome of *A. hydrophila* has been sequenced and was reported in 2006. Several laboratories are exploring specific genetic loci for potential virulence factors.

For Consumers: A Snapshot

This bacterium can cause different forms of illness. It's thought that people can get diarrhea from eating or drinking food or water contaminated with the bacterium, although this isn't believed to be common. In otherwise healthy people, the diarrhea usually is watery and goes away by itself in a few days. In some people, this form of the illness may become more severe, with worse diarrhea that may contain blood and mucus and may last for weeks. People with weak immune systems are especially at risk of getting the more severe type and of having the infection spread to other parts of the body. Those people, especially, and anyone who has diarrhea for long periods, should get treatment from a health professional. In the environment, the bacterium lives in freshwater and somewhat salty water. It has been found often in fish and shellfish, but also has been found in meats and various kinds of produce. Follow basic food-safety tips, which include thorough washing of fruits and vegetables and thorough cooking of meats and fish, to help protect yourself from the foodborne form of the illness caused by this bacterium. (Another form of the illness isn't caused by eating contaminated food or water, but by infection of an open wound – for example, from swimming. That form of the illness can be very serious, but isn't covered in this chapter, since this book is about illnesses from eating or drinking contaminated food.

2. Disease

- **Mortality**: For gastroenteritis, the mortality rate is not known. The mortality rate for septicemia caused by *Aeromonas* may be 33% or higher. (Infections that were not foodborne – skin or soft tissue infections caused by *Aeromonas*, particularly in immunocompromised people with conditions such as liver disease or malignancy – can result in mortality rates of 60% to 75%.)

- **Infective dose**: The infective dose of this organism is unknown, but SCUBA divers who have ingested small amounts of water have become ill, and *A. hydrophila* has been isolated from their stools. Although the organism possesses several virulence factors that could cause human illness, volunteer feeding studies using healthy adults and high concentrations of organism (10^4 to 10^{10} cells) have failed to elicit human illness. However, an outbreak associated with shrimp salad contaminated with *A. hydrophila*, at approximately 10^7 cfu/gm of food, has been reported.

- **Onset:** The incubation period associated with gastroenteritis is unknown (as strong challenge studies of volunteers and an animal model are lacking), although the onset of diarrhea appears to be greater than 24 hours.

- **Illness / complications:** The link between *A. hydrophila* and human gastroenteritis has not yet been firmly established. The link between the pathogen and disease in humans is based mostly on epidemiologic data.

- Clinically, different types of ga**s**troenteritis are associated with *A. hydrophila,* including mild diarrhea to a *Shigella*-like dysenteric illness characterized by loose stools containing blood and mucus, and colitis. In people with weak or impaired immune systems, diarrhea can be chronic and severe. Rarely, the dysentery-like syndrome is severe.

- In people with impaired immune systems, *A. hydrophila* may spread throughout the body and cause systemic infections. Examples of those at risk include people with cirrhosis or various kinds of cancer and those treated with immunosuppressive drugs or who are undergoing cancer chemotherapy. *A. caviae* and *A. veronii* biovar *sobria* also may cause enteritis and, in immunocompromised people or those with malignancies, septicemia. Along with *hydrophila,* these bacteria account for the majority of human clinical isolates of *Aeromonas*.

- Aside from foodborne infections, *Aeromonas* spp. are well documented as causative agents of wound infection, usually linked to water-related injuries or aquatic recreational activities.

- **Symptoms**: Range from mild diarrhea to dysentery-like symptoms, including blood and mucus in the stool, to symptoms of septicemia.

- **Duration**: The gastroenteritis associated with the milder form of the disease is usually self-limiting, with watery diarrhea present for a few days to a few weeks. However, people with the severe dysentery-like syndrome may have symptoms for several weeks.

- **Route of entry**: The foodborne form of the illness results from ingestion of a sufficient number of the organisms in foods (from animal origin, seafood, or produce) or water. (As noted, infection resulting not from ingestion, but from open wounds, may lead to tissue infections and septicemia.)

- **Pathway:** Illness is thought to be caused by toxins (aerolysin) and other virulence factors produced by aeromonads.

3. Frequency

The relative frequency of *A. hydrophila* disease in the United States is unknown, since efforts to ascertain its true incidence have only recently been attempted. Most cases have been sporadic, rather than associated with large outbreaks.

4. Sources

A. hydrophila frequently has been found in fish and shellfish. It has also been found in market samples of meats (beef, pork, lamb, and poultry) and produce.

5. Diagnosis

A. hydrophila can be cultured from stools or from blood by plating the organisms on an agar medium containing sheep blood and the antibiotic ampicillin. Ampicillin prevents the growth of most competing microorganisms. The species identification is confirmed by a series of biochemical tests. The ability of the organism to produce the enterotoxins believed to cause the gastrointestinal symptoms can be confirmed by tissue-culture assays.

6. Target Populations

All people are believed to be susceptible to gastroenteritis from *Aeromonas*, although it is most frequently observed in very young children. People with impaired immune systems or underlying malignancy are susceptible to the more severe or systemic infections.

7. Food Analysis

A. hydrophila can be recovered from most foods by direct plating onto a solid medium containing starch as the sole carbohydrate source and supplemented with antibiotics, such as ampicillin, to reduce the growth of most competing microorganisms. PCR-based assays have been developed to detect pathogenic *A. hydrophila* and differentiate non-pathogenic strains from pathogenic isolates.

8. Examples of Outbreaks

For more information on recent outbreaks, see the Morbidity and Mortality Weekly Report from the Centers for Disease Control and Prevention.

9. Other Resources

- Loci index for genome *Aeromonas hydrophila*
- GenBank Taxonomy database
- A recent review of *Aeromonas* infections is available in a paper by Janda and Abbott, 2010: The Genus Aeromonas: Taxonomy, Pathogenicity, and Infection. Clinical Microbiological Reviews. Volume 23. Pages 35-73.
- The EPA provides information about waterborne *Aeromonas* and the Unregulated Contaminant Monitoring Program**.**
- EPA Office of Water, March 2006. Aeromonas: Human Health Criteria Document.

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Plesiomonas shigelloides

1. Organism

Plesiomonas shigelloides is a Gram-negative, motile, non-sporulating, oxidase-positive, rod-shaped bacterium that has been found in many aquatic ecosystems. This bacterium has been isolated from freshwater (ponds, streams, rivers), estuarine water, and marine environments. The pathogen has been isolated from warm-blooded and cold-blooded animals, including freshwater fish and shellfish, and from many types of animals, including cattle, goats, swine, cats, dogs, monkeys, vultures, snakes, and toads.
P. shigelloides is not considered a commensal organism found in humans.

The ingested *P. shigelloides* organism does not always cause illness in the host animal, but may reside temporarily as a transient, noninfectious member of the intestinal flora. It has been isolated from the stools of patients with diarrhea, but is also sometimes isolated from healthy individuals (0.2% to 3.2% of the population).

Under laboratory conditions, *P. shigelloides* is able to grow at temperatures between 8°C and 45°C, with an optimal range from 25°C to 35°C. Growth is reduced in low temperatures (less than 10°C), pH less than 4.5, and in salt (NaCl) above 5%. *P. shigelloides* cells are killed by pasteurization.

For Consumers: A Snapshot

This bacterium is found in freshwater (rivers, streams, and ponds, for example) and water used for recreation. It can cause illness through unsanitary drinking water, contaminated seafood, and fruits and vegetables contaminated by unsanitary water. It takes a lot of these bacteria to cause an illness. When it does occur, the illness starts in about a day or two, and most otherwise healthy people have mild, watery diarrhea and get better in as little as one day or within about a week. Other symptoms may occur, such as chills, fever, cramps, and vomiting. In more severe cases, the diarrhea may last as long as 3 weeks and may be greenish-yellow, foamy, and a little bit bloody, and may contain mucus. Severe cramps and vomiting may occur, and a person may lose a lot of body fluid (become dehydrated), which needs to be replaced, along with certain minerals. Those people, especially, should see a health professional, to get treatment. Elderly and very young people and people with other, serious medical conditions or weak immune systems are more at risk of getting this illness than are others. In very severe cases, the infection could spread to other parts of the body, including the brain. To help protect yourself from this illness, don't use unsanitary water for drinking, washing foods, or anything else; cook your seafood well; wash fruits and vegetables in running water – follow basic food-safety tips.

2. Disease

This pathogen has been associated with human diarrheal diseases, but the number of cases that directly link *P. shigelloides* as a definite cause of human disease from ingestion of contaminated foods is quite low. There have been several putative virulence factors identified in this pathogen, but solid data to relate their functions to pathogenesis have not been firmly established.
P. shigelloides may be considered a low-potential pathogen.

- **Mortality**: Not very common, although one fatality of a newborn has been reported.

- **Infective dose:** The infective dose is presumed to be quite high; at least greater than 1 million organisms.

- **Onset**: Symptoms may begin 20 to 50 hours after consumption of contaminated food or water.

- **Illness / complications:** *P. shigelloides* gastroenteritis usually is a mild, self-limiting infection, although a more severe, dysenteric form of gastroenteritis may occur. Infected people may also exhibit other symptoms, such as severe abdominal pain, cramping, nausea, vomiting, low-grade fever, chills, headache, and some dehydration.

  There is a paucity of reports on extra-intestinal diseases caused by *P. shigelloides*, but the organism has been shown to be responsible for septicemia, bacteremia, meningitis, septic arthritis, osteomyelitis, peritonitis, cellulitis, and pneumonia. Extraintestinal complications, regardless of the organism's portal of entry (e.g., open wounds), may occur more frequently in people who are immunocompromised or seriously ill with cancer, blood disorders, or hepatobiliary disease. Neonates with meningitis most likely were infected by vertical transmission; mortality rate approaches 80%. Septicemia is more commonly found in adults.

- **Symptoms**: Symptoms may include fever, chills, abdominal pain, nausea, diarrhea, and/or vomiting. Diarrhea is watery, non-mucoid, and non-bloody. In severe cases, it may be greenish-yellow, foamy, and blood-tinged, and may contain mucus and polymorphonuclear leukocytes, and some patients experience severe abdominal cramps, vomiting, and some level of dehydration.

- **Duration**: Generally about 1 to 7 days, but diarrhea can last as long as 21 days.

- **Route of entry**: Oral; ingestion of contaminated water or foods causes the foodborne illness (vs. infection of open wounds).

- **Pathway**: The pathogen has several putative virulence factors, but the exact role of these proteins has not been elucidated. *P. shigelloides* synthesize toxins, including heat-stable toxins, heat-stable and heat-labile enterotoxin, cholerae-like toxins, hemolysin, cytotoxins, and iron sequestration in host cells. Other reported factors that indicate pathogenicity potential include cell adhesion, cell invasiveness, and apoptotic Caco-2 cell death. A direct link between these virulence factors, combined with the paucity of epidemiologic data, reflect the current debate about the true pathogenic potential of *P. shigelloides.*

3. Frequency

The rate of *P. shigelloides* infection in the United States is unknown. Gastrointestinal *P. shigelloides* illness in healthy people may be so mild that they do not seek medical treatment. Most *P. shigelloides* strains associated with human gastrointestinal disease have been from stools of diarrheic patients living in tropical and subtropical areas. Such infections are rarely reported in the U.S. or Europe because of the self-limiting nature of the disease. Most cases reported in the U.S. involve the elderly and very young and people who have pre-existing health problems, such as cancer or sickle-cell anemia, or are immunocompromised.

4. Sources

Most human *P. shigelloides* infections are suspected to be waterborne, occur in the summer months, and correlate with environmental contamination of freshwater (e.g., rivers, streams, ponds). The organism may be present in unsanitary water that has been used as drinking water or recreational water, or water used to rinse foods that are consumed without cooking or heating. The usual route of transmission of the organism in sporadic or epidemic cases is by ingestion of contaminated water, raw shellfish, or improperly cooked or raw foods. Additional aquatic sources include crabs, fish, and oysters.

5. Diagnosis

The pathogenesis of *P. shigelloides* infection is not known. The organism is suspected of being toxigenic and invasive. Its significance as an enteric (intestinal) pathogen is presumed because of its predominant isolation from stools of patients with diarrhea. It is identified by common bacteriological analysis, serotyping, and antibiotic sensitivity testing.

6. Target Populations

Anyone is susceptible to infection. Infants, children, and chronically ill people are more likely to experience protracted illness and complications. The pathogen also is associated with traveler's diarrhea.

7. Food Analysis

P. shigelloides may be recovered from food and water by methods similar to those used for stool analysis. The keys to recovery, in all cases, are selective agars that enhance the survival and growth of these bacteria over the growth of the background microflora. Suitable media to isolate *P. shigelloides* from foods include xylose-sodium deoxycholate-citrate, inositol-brilliant green, and Plesiomonas agars. Identification following recovery may be completed in 12 to 24 hours. PCR-based assays that have been reported in the literature can specifically detect *P. shigelloides*; genetic targets include the 23S rRNA gene and the hugA gene.

8. Examples of Outbreaks

For information about recent outbreaks, see the Centers for Disease Control and Prevention's Morbidity and Mortality Weekly Reports for information about *Plesiomonas outbreaks*.

9. Other Resources

- Loci index for genome *Plesiomonas shigelloides*
- Additional information on *Plesiomonas shigelloides* can be found in *Folia Microbiologica*.
- GenBank Taxonomy database

Bad Bug Book

Foodborne Pathogenic Microorganisms and Natural Toxins

Miscellaneous bacterial enterics

1. Organisms

Miscellaneous enterics. Gram-negative genera, including *Klebsiella, Enterobacter, Proteus, Citrobacter, Aerobacter, Providencia, Serratia*

These rod-shaped enteric bacteria have been suspected of causing acute and chronic gastrointestinal disease. The organisms may be recovered from natural environments, such as forests and freshwater, and from farm produce, where they reside as normal microflora. They also may be recovered from stools of healthy people with no disease symptoms.

The relative proportion of pathogenic to nonpathogenic strains is unknown. Some of these bacteria are associated with food spoilage, such as *Klebsiella oxytoca*; *Serratia marcescens*; *Aeromonas*; *Proteus*; *Pantoea*; previously, *Enterobacter agglomerans*; and *Citrobacter freundii*.

For Consumers: A Snapshot

These bacteria are often found in healthy people and often don't cause illness if they contaminate food and are eaten – but sometimes they do, although it's not completely clear how or why. Some reasons may be that bacteria have variations in their genes, and, for the most part, their genes often undergo changes. Those changes sometimes affect whether or not the bacteria can cause illness and the severity of the illness. Whether or not they cause illness also may depend on the people who eat them – their health, their own genetic make-up, and/or how much of the bacteria they eat. The illness these bacteria are thought to sometimes cause if they contaminate food is gastroenteritis – watery diarrhea and other symptoms that may include nausea, vomiting, cramps, pain, fever, and chills. Children in countries with poor sanitation are thought to get sick from these bacteria more often than do other people. Always following basic food-safety tips can help protect you from getting sick from these and other bacteria and viruses that can contaminate food.

Klebsiella species are ubiquitous in nature and commonly are in water and food. In regard to foods, *Klebsiella pneumoniae* may be considered as an enteropathogen, since this organism produces heat-stable and heat-labile (HT and ST) enterotoxins and has been associated with a few foodborne cases, usually associated with the presence of very high numbers of the pathogen. In some cases, colonization of the gastrointestinal tract is the initial stage for a systemic infection. *Klebsiella* also can be found in unpasteurized milk.

Proteus are more commonly sources of urinary tract and wound infections and of meningitis in neonates and infants than of gastroenteritis. They are a source of nosocomial infections. With regard to foods, *Proteus* can metabolize amino acids found in meats to produce compounds that can cause putrefaction. In fish, such as tuna, *Proteus* is considered a histamine-producing microbe and under such circumstances can generate scombroid poisoning.

Serratia species are not members of the bacterial populations found in the human intestinal tract, unlike *Klebsiella* species. *Serratia* are opportunistic pathogens and commonly are found to be sources of nosocomial infections. Antibiotic-resistant strains, particularly in immunocompromised patients, present a challenge to treatment.

Enterobacter species can be found in many environments, such as water, soil, sewage, and vegetables. *Enterobacter sakazakii* has been associated with powdered infant formula and has

been linked to meningitis and necrotizing enterocolitis, and can cause death. *Enterobacter sakazakii* has been moved to the genus *Cronobacter* and is described in a separate chapter. *Enterobacter cloacae* and *Enterobacter aerogenes* are opportunistic pathogens widely distributed in nature and have been found in dairy products, vegetables, spices, and meats.

Citrobacter freundii is another opportunistic pathogen, but also is a resident of the human gastrointestinal tract. This pathogen can be isolated from various types of foods, including meats, spices, and freshwater fish. Some foodborne outbreaks have been linked to enterotoxigenic *C. freundii*; patients typically exhibit diarrhea. This pathogen may also produce a Shiga-like toxin and produce hemolytic uremic syndrome.

Providencia species usually are associated with infections of the urinary tract, but they also can colonize the gastrointestinal tract.

2. Disease

- **Mortality**: Unknown; see last sentence of Illness / complications section, below.
- **Infective dose:** Unknown.
- **Onset**: Acute gastroenteritis may begin within 12 to 24 hours of ingesting the contaminated food or water.
- **Illness / complications:** These genera are thought to occasionally and sporadically cause acute or chronic gastroenteritis. As with other pathogens, people are asymptomatic in some cases and may be considered carriers. Malnourished children (1 to 4 years old) and infants with chronic diarrhea develop structural and functional abnormalities of their intestinal tracts, resulting in loss of ability to absorb nutrients. Death is not uncommon in these children and results indirectly from the chronic toxigenic effects that produce the malabsorption and malnutrition.
- **Symptoms:** Acute gastroenteritis may include vomiting, nausea, fever, chills, abdominal pain, and watery (dehydrating) diarrhea. Chronic diarrheal disease is characterized by dysenteric symptoms: foul-smelling, mucus-containing, diarrheic stool, with flatulence and abdominal distention. The chronic disease may continue for months and require antibiotic treatment.
- **Duration**: Otherwise healthy people typically recover quickly and without treatment from the acute form of gastrointestinal disease.
- **Route of entry**: Oral.
- **Pathway:** Both the acute and chronic forms of these illnesses are suspected to result from the elaboration of enterotoxins. These organisms may become transiently virulent by gaining mobilizeable genetic elements from other pathogens. For example, pathogenic *Citrobacter freundii* that elaborates a toxin identical to *E. coli* heat-stable toxin has been isolated from the stools of ill children.

3. Frequency

These pathogens are not reportable to the Centers for Disease Control and Prevention (CDC); thus, the frequency with which they cause illness is not known. For some of the pathogens in this chapter, no strong link to foodborne illness has been made; for example, to *Proteus*.

4. Sources

These bacteria have been recovered from dairy products, raw shellfish, and fresh, raw vegetables. Some of these organisms also occur in soils used for crop production and waters in which shellfish are harvested and, therefore, may pose a health hazard.

5. Diagnosis

Recovery and identification methods for these organisms from food, water, or diarrheal specimens are based on the efficacy of selective media and results of microbiologic and biochemical assays. The organism's ability to produce enterotoxin(s) may be determined by PCR-based assays.

6. Target Populations

All people may be susceptible to pathogenic forms of these bacteria. Acute gastrointestinal illness may occur more frequently in undeveloped areas of the world. The chronic illness is common in malnourished children living in unsanitary conditions in tropical countries. Protracted illness is more commonly experienced by the very young.

Immunocompromised people may be more susceptible to illness from these pathogens than are immunocompetent people, but that may also depend on the bacterial strain (how virulent it is) and how much of it is consumed.

7. Food Analysis

These strains are recovered by standard selective and differential isolation procedures for enteric bacteria. Biochemical and *in vitro* assays may be used to determine species and pathogenic potential. These human pathogens are very minor etiologic agents of foodborne diseases, and they may easily be overlooked by a food microbiology laboratory.

8. Examples of Outbreaks

Klebsiella isolated from contaminated hamburger.

9. Other Resources

Loci index for genome *Klebsiella* spp.

Enterobacter spp.

Proteus spp.

Citrobacter spp.

Providencia spp.

Serratia spp.

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Foodborne Pathogenic Microorganisms and Natural Toxins

Francisella tularensis

1. Organism

Francisella tularensis is a Gram-negative, non-motile, non-sporulating coccobacillus that can cause severe, life-threatening illness in humans. It is a facultative intracellular bacterium (i.e., it is capable of growth in both microbiological media and in eukaryotic host cells). Classification of *F. tularensis* consists of four closely associated subspecies: *tularensis* (also called Type A), *holarctica* (also called Type B), *novicida*, and *mediasiatica*.

F. tularensis is a fastidious, slow-growing bacterium that requires extended periods of time and enriched media for propagation. Nevertheless, it is resilient and can live for months in soil, vegetation, and water, which can act as a source of contamination and infection for animals and humans. *F. tularensis* has been shown to remain viable in specific foods and water for long periods and can survive the acidic environment of the stomach. However, it is less tolerant to high temperatures than are other, more traditional enteric bacterial pathogens.

2. Disease

- Infection with *F. tularensis* causes the disease tularemia. Idiomatic names have included rabbit fever, deerfly fever, hare fever, and lemming fever. The illness is contracted via the bite of an infected arthropod (insect), handling of contaminated animal products, inhalation of contaminated aerosols, and ingestion of tainted food (including animals and milk) or water. The illness is treatable, particularly in the early stages, with antibiotics to which the organism is sensitive.

For Consumers: A Snapshot

The bacterium *Francisella tularensis* causes a disease called tularemia (nicknamed "rabbit fever"). Tularemia can take different forms, depending on how the bacterium enters the body. If it enters through the mouth when a person eats or drinks contaminated food or water, it can cause tularemia that affects the throat or intestines, although this is an uncommon form of the disease. Symptoms of this type range from mild to severe in otherwise healthy people, and it rarely causes death. In the more serious cases, untreated throat infection may spread to vital organs (such as the lungs, brain, or liver), and may cause extensive bowel damage, with bleeding and infection of the bloodstream, especially in people with weak immune systems. People can develop tularemia of the throat or intestines by eating undercooked meat from an infected animal (particularly rabbits) or drinking contaminated water. Eating food or drinking water contaminated by animal waste, such as rodent droppings, also can cause this form of tularemia and many other diseases. Cooking food well is one of the safety tips that can help protect you from getting this form of tularemia, especially if you eat the kind of wild animals known to be carriers, such as rabbits. Other forms of tularemia can come from inhaling the bacterium; from the bite of certain insects, including some kinds of ticks; and from an open wound that comes into contact with an infected animal. Inhaling the bacterium is of particular concern, because it could lead to very serious infection in the lungs. For *any* form of tularemia, getting immediate medical help is very important, to get the right kind of antibiotics to keep the infection from progressing.

- **Mortality**: Mortality rates from infection are determined largely by the virulence of the subspecies involved and the type of tularemia contracted. Tularemia obtained from ingestion of contaminated food or water (oropharyngeal and gastrointestinal tularemia) are not typically associated with a high fatality rate; however, oropharyngeal tularemia can progress to the more fulminant secondary pneumonic tularemia from bacteremic spread to the lungs, resulting in a high rate of mortality. In addition, severe *g*astrointestinal tularemia can result in extensive bowel ulceration, leading to a fatal outcome. Typhoidal tularemia produced from *F. tularensis* subspecies *tularensis* (i.e., *F. tularensis* Type A) infection can carry a fatality rate as high as 60% in untreated cases, whereas the most common type of tularemia (ulceroglandular tularemia) displays a fatality rate of less than 3%.

- **Infective dose:** As few as 10 organisms are known to be sufficient to initiate disease via inhalation of the organism, making this bacterium one of the most infectious microorganisms known. Larger doses of bacteria, however (approximately 1 million to 10 million), are required to initiate infection through ingestion.

- Onset of symptoms generally appears after an incubation period of 3 to 6 days. However, symptoms can emerge within a day or as long as a few weeks, depending on the initial dose, route of exposure, and virulence of the infecting strain.

- **Illness / complications:** Subspecies *tularensis* (i.e., subspecies Type A) and subspecies *holarctica* (Type B) cause the majority of human disease. Type A is the most virulent subspecies and has the highest mortality rate. Type B causes a milder form of disease that is rarely fatal. Subspecies *mediasiatica* also produces a less severe infection. Subspecies *novicida* is primarily associated with disease in immunocompromised humans.

  There are many forms of tularemia, including pulmonary, gastrointestinal, oropharyngeal, typhoidal, oculoglandular, and ulceroglandular. The type of tularemia contracted by humans depends largely on the infecting strain, dose, and route of inoculation.

  Oropharyngeal tularemia can develop when ingested bacteria colonize the throat, leading to symptoms that include exudative pharyngitis and necrotic cervical adenopathy. Ingested bacteria can also colonize the intestines, resulting in gastrointestinal tularemia, with symptoms ranging from mild diarrhea to severe bowel damage.

  However, the most common form of tularemia is the ulceroglandular type, contracted from the bite of an infected insect (tick, deerfly) or from handling contaminated materials. A sudden onset of chills, fever, and headaches occurs after 3 to 6 days of exposure, with an ulcer appearing at the site of entry. Bacteria can enter the lymphatics and reside in regional lymph nodes, leading to their enlargement. Subsequent dispersal to other organs, including the liver, spleen, lungs, kidneys, and central nervous system, can occur.

  The most acute, deadly form of the disease results when contaminated aerosols are inhaled, leading to pulmonary tularemia. This results in a high mortality rate among untreated cases.

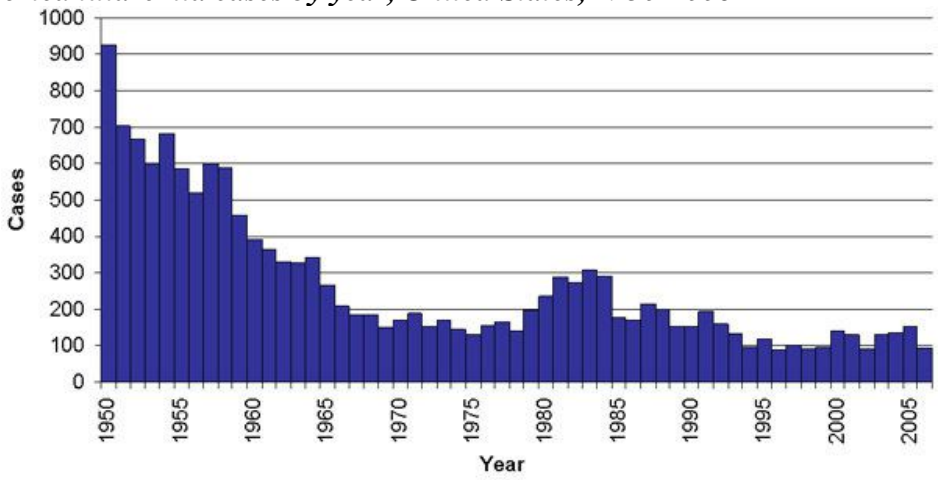
  Most forms of tularemia can develop into the more severe pulmonary form of the disease, if the bacteria spread to the lungs. In addition, bacterial meningitis can occur if the bacterium attains access to the cerebral and spinal meninges, which can lead to

permanent brain damage and death. Similarly, bacterial spread to the pericardium and bones can result in debilitating pericarditis and osteomyelitis, respectively.

- **Symptoms**: See above.
- **Duration**: Symptoms and their duration vary according to the type of tularemia contracted and the virulence of the strain.
- **Route of entry**: Oral, inhalation, insect bite, or direct contact with contaminated objects.
- **Pathway:** *F. tularensis* infection and disease symptoms are promoted by the organism's ability to invade, survive, and multiply within various host cells, particularly macrophages. The intracellular bacterium utilizes a unique mechanism to cripple the macrophages' ability to digest and eliminate the bacterium, thus allowing for further replication and initiation of infection.

3. Frequency

Reported tularemia cases by year, United States, 1950-2006



Centers for Disease Control and Prevention

Oropharyngeal and gastrointestinal tularemia are extremely rare in the United States and occur primarily in Europe. The incidence of tularemia worldwide is unknown. However, outbreaks have been reported in many countries, including Sweden, Japan, Spain, Kosovo, Turkey, and the U.S. Reported cases of the disease in the U.S. have steadily decreased during the 20th century. However, the true frequency may be much higher, given that many of the illnesses may go undiagnosed, due to the relatively mild symptoms elicited from some of the less virulent strains. The incidence of foodborne tularemia in the U.S. is unknown.

4. Sources

Among foods, milk and undercooked meats from infected animals (particularly rabbits and hares) have been implicated as vehicles for the transmission of *F. tularensis*. However, outbreaks often occur from consumption of food contaminated with infected rodent droppings and water contaminated with infected animal carcasses.

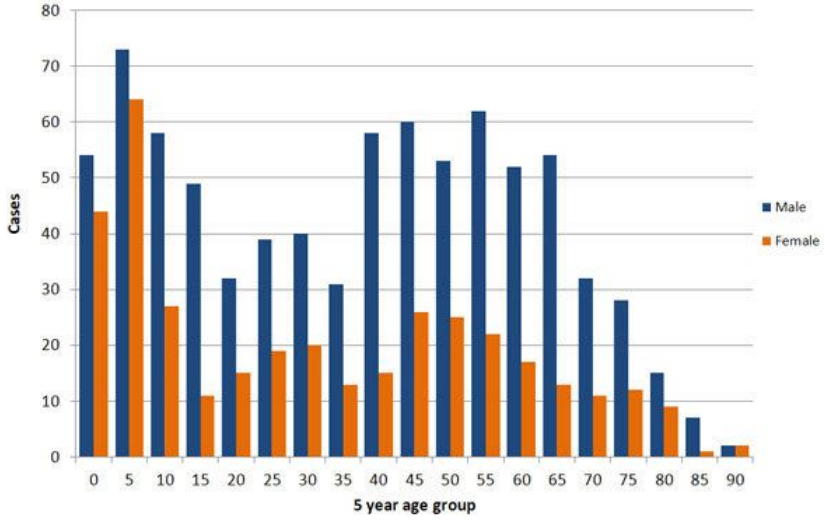
5. Diagnosis

Real-time PCR, direct fluorescent antibody (DFA), and ELISA-based techniques have been used for presumptive identification from clinical samples. However, confirmatory detection requires culturing of the organism on enriched media. Unfortunately, *F. tularensis* is a slow-growing organism on media and can take several days for visible growth to appear. Moreover, *F. tularensis* is often outcompeted for growth by other, faster-growing background microbial flora that are often present on environmental and food samples.

6. Target Populations

All age groups are susceptible to tularemia.

Reported tularemia cases by age and sex, United States, 2001-2010



Centers for Disease Control and Prevention

7. Food Analysis

Detection of *F. tularensis* by culture in food matrices is difficult, due to the slow-growing and fastidious nature of the organism. A new method that selectively isolates and enriches *F. tularensis* from select food matrices, using macrophage monolayers with subsequent identification using real-time PCR, has recently been developed.

8. Examples of Outbreaks

An outbreak of pulmonary tularemia occurred on Martha's Vineyard, in the summer of 2000, and involved 15 people. This was the second reported outbreak involving the pulmonary form of tularemia in the U.S. As noted, oropharyngeal and gastrointestinal tularemia are rare in the U.S. and occur primarily in Europe. One of the largest outbreaks of oropharyngeal tularemia occurred from 1999 to 2000, in Kosovo, where a total of 327 serologically confirmed cases were observed. In a 1998 – 2003 outbreak, in Bulgaria, 235 confirmed cases of oropharyngeal tularemia were detected.

For more information about recent outbreaks see Morbidity and Mortality Reports from CDC.

9. Other Resources

- The CDC provides information on emergency preparedness and responses regarding *Francisella tularensis*.

- Loci index for *Francisella tularemia*.

Pathogenic *Escherichia coli* Group

Introduction

Escherichia coli is one of the predominant enteric species in the human gut and, as part of the normal intestinal flora, some of these species provide many health benefits to the host; for example, they prevent colonization of the gut by harmful pathogens. However, there are small groups of *E. coli*, sometimes referred to as enterovirulent *E. coli*, diarrheagenic *E. coli*, or more commonly, pathogenic *E. coli*, that can cause severe diarrheal diseases in humans.

Currently, there are six recognized pathogenic groups: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC). Of these, the first four groups are well known to be transmitted via contaminated food or water; EHEC, especially, are often implicated in major foodborne outbreaks worldwide.

Pathogenic *E. coli* are generally grouped based on their virulence properties or factors that they carry. However, some groups can share similar virulence traits. For instance, both EPEC and EHEC produce intimin protein, which allows the pathogen to attach to intestinal cells. Also, many of the virulence genes carried by these pathogenic *E. coli* groups reside on mobile genetic elements and can be transferred. As an example, the *E. coli* strain of serotype O104:H4 that caused a large outbreak in Germany, in 2011, produced Shiga toxin, characteristic of EHEC – but, genetically, the strain was an enteroaggregative *E. coli* (EAEC). Historically, EAEC have been known to cause persistent diarrhea in underdeveloped countries, but seldom have been implicated in major foodborne incidents. Hence, the O104:H4 strain that caused this outbreak appeared to be an EAEC strain that had acquired the ability to produce Shiga toxin.

The following four chapters are descriptions of pathogenic *E. coli* that are most often transmitted via contaminated food or water.

Bad Bug Book

Foodborne Pathogenic Microorganisms and Natural Toxins

Enterotoxigenic *Escherichia coli* (ETEC)

1. Organism

Enterotoxigenic *Escherichia coli* (ETEC) are highly motile, Gram-negative, rod-shaped bacteria. They are characterized by production of several virulence factors, including both heat-labile (LT) toxin and heat-stable (ST) toxins, as well as several colonization-factor antigens.

2. Disease

ETEC causes gastroenteritis in humans and is best known as the causative agent of travelers' diarrhea. It is also an important cause of diarrhea in infants, in less-developed countries.

- **Mortality**: The World Health Organization attributes 380,000 deaths (worldwide) to ETEC, mostly among children, each year.
- **Infective dose:** Volunteer feeding studies showed that a high dose, ranging from 10 million to 10 billion ETEC cells, may be needed to cause an infection in adults. Children may be affected by a smaller dose.
- **Onset**: Usually 26 hours after ingestion of contaminant, but can range from 8 to 44 hours.

For Consumers: A Snapshot

This chapter is about the bacterium *E. coli*, but not the kind you've heard about in news reports about outbreaks. That kind of *E. coli* sometimes causes kidney failure and death, but the kind in this chapter, called ETEC for short, causes traveler's diarrhea. People in the U.S. usually don't get ETEC infections, unless they travel to areas of the world with poor sanitation. In most people, the illness goes away by itself, after a few days of bowel movements that look like rice-water, and cramps, perhaps a low fever, and nausea. But some people, especially infants and people with weak immune systems, can develop a severe illness more like cholera, which lasts up to 19 days. For these people, especially, getting treatment is very important. They can lose so much fluid from diarrhea that it upsets the chemical balance of their blood, which can lead to heartbeat disturbances and may even lead to death. In the U.S., this doesn't happen often, but in countries with poor sanitation, ETEC is a major cause of infant death. Contaminated water often is the source of ETEC, and that water or an infected food handler can contaminate food. Once it gets inside the bowels, the bacterium produces a toxic substance that causes the illness. You can help protect yourself from ETEC by drinking bottled water if you travel outside the U.S. and following basic food-safety tips.

- **Disease / complications:** Illness from ETEC is usually self-limiting, mild, and brief. However, some severe forms last longer and resemble cholera, with up to five or more daily passages of rice-water-like stools that result in severe dehydration. Antibiotic treatment usually is not required in ETEC infections, but seems to be effective in reducing the duration and severity of illness. In infants and elderly and debilitated patients, particularly, appropriate electrolyte replacement therapy may be necessary.
- **Symptoms**: Infection is characterized by sudden onset of diarrhea that is watery and without blood or mucus, rarely accompanied by high fever or vomiting. Other symptoms include abdominal cramps, low-grade fever, nausea, and malaise.

- **Duration of symptoms**: Most cases last a few days; however, severe forms can last up to 19 days.
- **Route of entry**: Oral.
- **Pathway**: After ingestion, ETEC colonizes the small intestine, where the toxins that induce fluid secretion are produced.

3. Frequency

ETEC outbreaks are infrequent in the United States, but infections are a more common occurrence among travelers to foreign countries. ETEC infections are endemic in many developing tropical countries and areas with poor hygiene standards. Infections are more prevalent in the warmer, wet months.

4. Sources

Most ETEC outbreaks are linked to consumption of contaminated food or water. ETEC is often found in feces of asymptomatic carriers, and humans appear to be the most likely source of ETEC. In 1975, a large outbreak affecting 2,000 people was traced to sewage-contaminated water at a national park. Contaminated well water in Japan and water supplies aboard cruise ships also have been implicated in ETEC outbreaks. Foodborne outbreaks of ETEC have occurred in restaurants and at various catered functions.

Examples of implicated foods include Brie cheese, curried turkey, mayonnaise, crabmeat, deli food, and salads. In most of these cases, foods became contaminated with ETEC via infected food handlers or through the use of contaminated water during preparation. ETEC infection does not appear to be transmitted by person-to-person contact, but some hospital infections have occurred and probably were caused by unsanitary conditions.

5. Diagnosis

During the acute phase of infection, large numbers of ETEC cells are excreted in feces. Since generic *E. coli* are also abundantly present in the bowels, ETEC strains can be differentiated from other *E. coli* by *in vitro* immunochemical assays, tissue culture, or gene probes and PCR assays specific for LT and ST toxin genes. Commercial kits that use antibodies to detect these toxins are also available**.**

6. Target Populations

Infants and travelers to underdeveloped countries are most at risk of ETEC infection. As with other infections, people with weak immune systems are more likely than others to suffer severe, even life-threatening cases.

7. Food Analysis

Presence of ETEC in foods can be determined by plating culture enrichment of food samples onto media that are selective and differential for *E. coli* and testing the isolates for presence of LT and ST toxins, using PCR or commercial kits that use antibodies specific to the toxins. Because of its high infectious dosage, ETEC analyses usually are not performed unless generic *E. coli* levels are very high.

8. Examples of Outbreaks

See the Centers for Disease Control and Prevention's Morbidity and Mortality Weekly Reports.

9. Other Resources

- Loci index for genome
- GenBank Taxonomy Database

Enteropathogenic *Escherichia coli* (EPEC)

1. Organism

EPEC are Gram-negative, rod-shaped bacteria. They are characterized by the presence of the locus for enterocyte effacement (LEE) pathogenicity island, which carries multiple virulence factors, including the *eae* gene that encodes for intimin and, together with the *tir* gene (intimin receptor), allows intimate adherence of EPEC to intestinal epithelial cells. In the 1940s and 1950s, EPEC was a frequent cause of infantile diarrhea in the United States. Currently, EPEC infections are less important in developed countries, but continue to be a common cause of diarrhea in developing countries, especially in children less than 2 years old.

For Consumers: A Snapshot

Many *E. coli* are harmless, but some cause serious illness if they contaminate foods and are eaten. This book covers four kinds of *E. coli* that cause foodborne illness. The one in this chapter, called "EPEC" for short, isn't the one that causes many of the outbreaks that make headlines in newspapers. (That kind, sometimes called "EHEC," includes *E. coli* 0157:H7, can cause problems like kidney failure, and is especially serious.) Although EPEC mostly affects countries with poor sanitation and has become less of a problem in countries like the U.S., it still can occur here. Over the decades, with advances in medicine and sanitation, the death rate from foodborne EPEC has dropped in developed countries. In a recent estimate of illness from contaminated food in the U.S., the Centers for Disease Control and Prevention listed no deaths from EPEC. Still, this illness should be taken seriously, as it occurs most often in children under age 2. The symptoms are often mild, but sometimes are severe, lasting for weeks or months. In the most severe cases, it can cause so much watery diarrhea that a child loses dangerous amounts of body fluid and minerals, and medical treatment is needed. Day-care centers and pediatric wards in countries with poor sanitation are often high-risk places for EPEC outbreaks. In those countries, bottle-fed babies seem to be at highest risk – but any food or fluid contaminated with feces (from someone infected who doesn't wash his or her hands after a bowel movement, for example) can spread the illness. To help protect yourself and others, follow basic food-safety tips. For tips about how to handle baby bottles safely, see the chapter called "*Cronobacter*," and look for the box called "For Consumers: A Snapshot."

2. Disease

The disease usually associated with EPEC is infantile diarrhea.

- **Mortality:** Mortality rates from 25% to 50% have been reported in the past. In developed countries, better treatment and medical facilities have greatly reduced mortality, but some deaths still occur.
- **Infective dose:** EPEC is highly infective in infants, and the dose is presumably very low. Adults, however, are not as susceptible. Volunteer feeding studies showed that 10 million to 10 billion cells are needed to cause diarrhea in adults, provided that gastric acid first has been neutralized by bicarbonate.
- **Onset:** Onset of diarrhea is often rapid, occurring as soon as 4 hours post ingestion of EPEC.

- **Illness / complications:** The diarrhea can be mild; however, the infection sometimes can be severe. Fluid and electrolyte imbalance may need to be corrected, to prevent dehydration.

- **Symptoms:** Profuse, watery diarrhea; vomiting; and low-grade fever.

- **Duration:** Diarrhea occasionally is protracted, lasting from 21 to 120 days.

- **Route of entry:** Oral.

- **Pathway:** After ingestion, EPEC adheres to intestinal mucosa and causes extensive disarrangement of the digestive-absorptive enzyme system, resulting in nutrient malabsorption.

3. Frequency

Foodborne outbreaks of EPEC are sporadic. Incidence varies on a worldwide basis, but countries with poor sanitation practices have the most frequent outbreaks. Many of these occur in day-care centers and pediatric wards.

4. Sources

Source(s) and prevalence of EPEC are controversial, because foodborne outbreaks are sporadic. Foods implicated in past EPEC outbreaks have included raw beef and chicken, but any food exposed to fecal contamination is strongly suspect. In the mid 1990s, an EPEC outbreak in Minnesota was traced to a buffet, but no specific food item was identified. In 1995, two outbreaks in France affected 59 people and were traced to mayonnaise, lettuce, and pickles.

5. Diagnosis

Diagnosis consists of culturing for *E. coli* from stools of infected people and testing the isolates for the ability to cause attachment and effacing (A/E) lesions on tissue culture cells. PCR assays can also be used to test the isolates for LEE genes, but since Enterohemorrhagic *E. coli* (EHEC) also carry LEE, the isolates also have to be tested for Shiga toxins (Stx). EPEC are distinguished from EHEC by the presence of LEE and absence of Stx.

6. Target Populations

EPEC infections most often occur in infants, especially those who are being bottle fed. Poor-quality water used to rehydrate infant formulae in underdeveloped countries may be the source of EPEC in bottle-fed infants.

7. Food Analysis

Presence of EPEC in foods can be determined by plating culture enrichment of food samples onto media that are selective and differential for *E. coli* and testing the isolates for EPEC traits by tissue culture or PCR. Shiga toxins (Stx) assays are also essential to distinguish EHEC from EPEC. EPEC are characterized by the presence of LEE and absence of Stx.

8. Examples of Outbreaks

Check the CDC's Morbidity and Mortality Weekly Reports for articles about outbreaks.

9. Resources

- Loci index for genome
- GenBank Taxonomy Database

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Foodborne Pathogenic Microorganisms and Natural Toxins

Enterohemorrhagic *Escherichia coli* (EHEC)

1. Organism

Like generic *E. coli*, toxin-producing Shiga-toxigenic *Escherichia coli* (STEC) are Gram-negative, rod-shaped bacteria, but are characterized by the production of Shiga toxins (Stx). Depending on the reference cited, there are 200 to 400 STEC serotypes, many of which have not been implicated in human illness; however, a subset of STEC called enterohemorrhagic *Escherichia coli* (EHEC), the topic of this chapter, includes only those that cause serious illness. Serotype O157:H7 is the prototypic EHEC strain.

For Consumers: A Snapshot

Most *E. coli* bacteria are harmless, but some produce a toxin (Shiga toxin) that can cause serious illness, including bloody diarrhea, blood-clotting problems, kidney failure, and death. Not all of the Shiga-producing *E. coli* can cause these problems, but the subset called enterohemorrhagic *E. coli* (EHEC) can. You might have heard news reports about these EHEC bacteria, such as *E. coli* O157:H7, when they've caused outbreaks of foodborne illness. EHEC outbreaks have been traced to many kinds of foods; for example, ground meats, **un**pasteurized ("raw") milk, unpasteurized fruit juice, lettuce, spinach, sprouts, and, more recently, commercially manufactured frozen cookie dough. Some people get the less serious form of the infection, which can range from no symptoms to diarrhea that starts out watery, then turns bloody. But the infection sometimes progresses into the life-threatening form of the illness that causes kidney failure and other problems, with children and people with weak immune systems being at especially high risk. Cooking ground beef well; washing raw fruits and vegetables under clean, running water; and ***not*** drinking **un**pasteurized ("raw") milk or eating certain cheeses made from it are some of the things you can do to help protect yourself.

Although O157:H7 is currently the predominant strain and accounts for ~75% of the EHEC infections worldwide, other non-O157 EHEC serotypes are emerging as a cause of foodborne illnesses. In the United States a group often referred to as the "big 6" (O111, O26, O121, O103, O145, and O45) accounts for the majority of the non-O157:H7 serotypes isolated from clinical infections and, therefore, is currently a focus of concern. However, other EHEC serotypes, such as O113, O91, and others, also can cause severe illness. As a result, the non-O157 EHEC serotypes of public health concern can change quickly, depending on outbreak incidents, and can vary with countries and geographic regions.

A recent example is the large outbreak, in 2011, that was centered in Germany, but also affected various other countries in the European Union. The pathogen was identified as an E. coli strain of serotype O104:H4 that produced Shiga toxin and, therefore, was thought to be an EHEC. However, genetic analysis showed that this pathogen had 93% genetic homology with a strain of Enteroaggregative *E. coli* (EAEC), which is known for causing persistent diarrhea in under-developed countries, but has seldom been implicated in major foodborne incidents. Hence, the O104:H4 strain that caused the outbreak appears to be an EAEC strain that acquired the ability to produce Shiga toxin.

Currently, it is difficult to determine which serotypes of *E. coli* are EHEC and equally challenging to predict the emergence of strains that can acquire the genes for Shiga toxin production or other virulence factors and so cause human illness. EHEC are characterized by:

- production of Stx, including Stx1 and/or Stx2. Stx1 is nearly identical to the toxin produced by *Shigella dysenteriae* Type I. There are many subtypes of both toxins, and some subtypes of Stx2 appear to be implicated in human illness. Stx2 is most often associated with severe sequelae, such as hemolytic uremic syndrome (HUS), which is characterized by acute renal failure.
- presence of LEE ("locus for enterocyte effacement"; pathogenicity island that encodes for intimin, a protein that enables bacterial attachment to epithelial cells).

There are also several other putative virulence factors, including enterohemolysin, but the role of these factors in pathogenesis remains undetermined.

2. Disease

- **Mortality**: Patients whose illness progresses to HUS have a mortality rate of 3% to 5%.
- **Infective dose**: The infective dose of EHEC O157:H7 is estimated to be very low, in the range of 10 to 100 cells. The infective dose of other EHEC serotypes is suspected to be slightly higher.
- **Onset**: Symptoms usually begin 3 to 4 days after exposure, but the time may range from 1 to 9 days.
- **Disease / complications:** Infections from EHEC may range from asymptomatic-to-mild diarrhea to severe complications. The acute symptoms are called hemorrhagic colitis (HC), characterized by severe abdominal cramps and bloody diarrhea, which may progress to such life-threatening complications as HUS or thrombotic thrombocytopenia purpura (TTP) – conditions that are most often associated with O157:H7, but that also can occur with other EHEC serotypes. About 3% to 7% of HC cases progress to HUS or TTP.

  Some evidence suggests that Stx2 and intimin are associated with progression to severe disease, such as HUS. Kidney cells have a high concentration of Stx receptors; hence, the kidney is a common site of damage. Some survivors may have permanent disabilities, such as renal insufficiency and neurological deficits.

  Antibiotic therapy for EHEC infection has had mixed results and, in some instances, seems to increase the patient's risk of HUS. One speculation is that antibiotics lyse EHEC cells, releasing more Stx into the host.
- **Symptoms**: Hemorrhagic colitis is characterized by severe cramping (abdominal pain), nausea or vomiting, and diarrhea that initially is watery, but becomes grossly bloody. In some cases, the diarrhea may be extreme, appearing to consist entirely of blood and occurring every 15 to 30 minutes. Fever typically is low-grade or absent.
- **Duration**: In uncomplicated cases, duration of symptoms is 2 to 9 days, with an average of 8 days.
- **Route of entry**: Oral (e.g., ingestion of contaminated food, water, or fecal particles).

- **Pathway**: After ingestion, EHEC attaches to intestinal epithelial cells via LEE-encoded factors and produces Stx that are internalized, activated, and can pass into the bloodstream to become systemic.

3. Frequency

There are about 63,000 cases of EHEC infections in the U.S. yearly, according to a report by the Centers for Disease Control and Prevention (CDC) . Ground beef and beef products continue to be implicated in most infections; however, contaminated produce increasingly has been implicated as a vehicle. As for STEC non-O157, the CDC estimates that 112,752 cases, per year, are attributed to foodborne illness in the U.S.

EHEC O157:H7 was first identified in an outbreak, in 1982, in which hamburgers from a fast-food restaurant were the vehicle. In 1991, hamburgers from fast-food restaurants were implicated in another outbreak, which affected about 700 people in four states. In the mid 1990s, a large outbreak was traced to unpasteurized juices. The largest O157:H7 outbreak on record took place in Japan; radish sprouts were implicated and about 10,000 people were affected. Since then, O157:H7 has been implicated in numerous outbreaks that involved lettuce, salads, various types of sprouts, and, in 2006, bagged spinach. In 2009, an O157:H7 outbreak in the U.S. was traced to frozen, raw cookie dough.

About a dozen non-O157:H7 EHEC outbreaks have been recorded in the U.S., but incidences may be underestimated due to lack of routine testing and appropriate testing methods.

4. Sources

Raw or undercooked ground beef and beef products are the vehicles most often implicated in O157:H7 outbreaks. Earlier outbreaks also implicated consumption of raw milk. O157:H7 can develop acid tolerance, as evidenced by infections in which acid foods (<pH4.6) were implicated, such as yogurt, mayonnaise, fermented sausages, cheeses, and unpasteurized fruit juices.

Various water sources, including potable, well, and recreational water, also have caused EHEC infections, as has contact with animals at farms or petting zoos.

Produce, including bagged lettuce, spinach, and alfalfa sprouts, increasingly is being implicated in O157:H7 infections.

Interestingly, infections in the U.S. by *non*-O157:H7 EHEC has been caused by many of these same vehicles, but, as of this writing, beef products have seldom been implicated.

Person-to-person transmission of infection is well documented.

Additional information is available from "*Escherichia coli* Serotype O157:H7: Novel Vehicles of Infection and Emergence of Phenotypic Variants," by Dr. Peter Feng, FDA. Emerging Infectious Diseases (1995) 1(2)

5. Diagnosis

Unlike generic *E. coli*, EHEC O157:H7 do not ferment the sugar sorbitol, so an effective method is to plate patient's bloody diarrhea samples onto sorbitol MacConkey medium to screen for sorbitol non-fermenting isolates. These are then typed serologically using antibodies to the O157 and the H7 antigens. However, as other EHEC serotypes are increasingly causing illness, clinical samples are now simultaneously tested for the presence of Stx using commercially-available antibody kits. Any STEC strains found are then serotyped and identified. There are also many PCR assays specific for Stx genes that may be used for screening clinical samples.

6. Target Populations

All people are believed to be susceptible to hemorrhagic colitis, but young children and the elderly are more susceptible and at higher risk for the illness to progress to more severe complications. Others with weak immune systems also are at risk, such as people with some chronic diseases or AIDS, and people on immunosuppressive medications; for example, some drugs used for arthritis and cancer chemotherapy.

7. Food Analysis

Presence of EHEC O157:H7 in foods can be determined by plating culture enrichment of food samples onto selective and differential media. Unlike typical *E. coli*, O157:H7 do not ferment sorbitol and are negative with the MUG assay, so these tests are commonly used to distinguish O157:H7 strains from other *E. coli* prior to serological testing for the O157 and H7 antigens and also for the presence of Stx genes by PCR. Molecular assays also exist that can specifically detect O157:H7 strains using unique mutational markers.

Detection of non-O157:H7 EHEC, however, is more complex, due to the lack of unique traits. For non-O157 EHEC, food enrichment is first screened for Shiga toxin using an antibody assay or for Stx genes by PCR. Enrichment cultures that are positive for Stx are plated on agar media, and multiple isolates are then tested for Stx genes, in order to obtain a pure culture isolate. These putative STEC isolates are then retested for virulence genes and their serotype determined. This process is both time-consuming and labor-intensive and may require screening hundreds of isolates.

There are numerous commercially-available kits to test for Stx, O157, and a few other EHEC serotypes. However, there are several Stx subtypes and many EHEC serotypes, and not all of these can be detected by commercial test kits. The *Escherichia coli* link to the FDA Bacteriological Analytical Manual, Chapter 4, provides a description of methods to test for common *E. coli*. Methods for EHEC and O157:H7 are described in Chapter 4a.

8. Examples of Outbreaks

For more information about recent outbreaks see the Centers for Disease Control and Prevention (CDC) Morbidity and Mortality Weekly Reports.

9. Other Resources

More information is available from the following sources.

- USDA (August 11 1998) – USDA Urges Consumers to Use Food Thermometer When Cooking Ground Beef Patties
- CDC – General information about *Escherichia coli* O157:H7
- Produce Handling and Processing Practices, from *Emerging Infectious Diseases*, CDC
- Risk assessment of *E. coli* O157:H7 in ground beef, from the USDA Food Safety and Inspection Service

Enteroinvasive *Escherichia coli* (EIEC)

1. Organism

EIEC is a Gram-negative, rod-shaped, enterotoxin-producing bacterium that closely resembles *Shigella*. Both are characterized by their ability to invade colonic epithelial cells. The genetic information required for the invasion phenotype is encoded within a 37 kilobase region on a virulence plasmid, which can vary in size from 180 kb in *S. sonnei* to 220 kb in *S. flexneri* and EIEC. There is a high degree of homology among these plasmids, and they are functionally interchangeable.

For Consumers: A Snapshot

Many *E. coli* are harmless, but some can cause serious illness from contaminated food. We cover four kinds of *E. coli* that cause foodborne illness in this book. The one described in this chapter, called "EIEC" for short, isn't the one that causes many of the outbreaks that make headlines in newspapers. (That kind, sometimes called "EHEC," includes *E. coli* O157:H7, can cause problems like kidney failure, and is especially serious.) The disease caused by EIEC usually begins as watery diarrhea, then progresses to mild dysentery – diarrhea that often contains blood and mucus. Other symptoms may include cramps, vomiting, fever, chills, and a general sense of not feeling well. In people who are otherwise healthy, the illness usually goes away by itself, without medical treatment. In a recent estimate of cases of illness from contaminated food eaten in the U.S., the Centers for Disease Control and Prevention listed zero deaths from EIEC. But if a case turns severe, a health professional should be consulted, so that fluid and important minerals lost due to diarrhea can be replaced, if need be. It's also important to know that EIEC can be passed not only by foods, but also by other people – for example, if infected people don't wash their hands well after having a bowel movement, then objects or other people they touch may become infected. To help protect yourself and others, follow good handwashing practices and other basic food-safety tips.

2. Disease

The illness caused by EIEC is a mild form of bacillary dysentery, similar to that caused by *Shigella* spp.

- **Mortality:** A recent estimate of domestically acquired foodborne illness in the United States, by the Centers for Disease Control and Prevention (CDC), lists a death rate of zero for diarrheagenic *E. coli* other than Shiga-toxigenic and enterotoxigenic *E. coli*.
- **Infective dose:** The infective dose of EIEC is thought to be in the range of 200 to 5,000 cells, somewhat higher than that of *Shigella*. The difference in the dose may depend on which virulence plasmid these pathogens harbor.
- **Onset:** The symptoms usually occur within 12 to 72 hrs after ingestion of contaminated food.
- **Illness / complications:** The illness generally is self-limiting, with no known complications.
- **Symptoms**: Mild dysentery; abdominal cramps, diarrhea, vomiting, fever, chills, and generalized malaise. Stools often contain blood and mucus.

- **Duration:** Usually resolves in 5 to 7 days.
- **Route of entry:** Oral. Person-to-person transmission can also occur.
- **Pathway:** The pathogenesis of EIEC is similar to that of *Shigella* species. The process begins with cellular invasion via endocytic vacuoles. Once internalized, the vacuoles are lysed, the bacteria multiply intracellularly, and spread laterally to other cells. EIEC also produce an enterotoxin, which may be involved in causing the watery diarrhea that precedes the dysentery symptoms associated with EIEC.

3. Frequency

EIEC outbreaks are not frequent in the U.S. However, it may be misidentified or confused with shigellosis; therefore, its actual prevalence may be underestimated.

4. Sources

No specific foods are frequently associated with EIEC infections. Infected humans are the only known reservoirs of EIEC; hence, any food contaminated with human feces from an ill individual, either directly or via contaminated water, can be infectious. Imported Camembert cheese was implicated in an epidemic of gastroenteritis caused by EIEC that affected 226 people in 96 outbreaks, in 1971. A tofu product contaminated with EIEC affected 670 people in Japan, in 1988. In 1994, a restaurant-associated EIEC outbreak in Texas, which affected 370 people, was traced to contaminated guacamole.

5. Diagnosis

Diagnosis consists of culturing for *E. coli* from stools of infected individuals and testing the isolates for invasiveness using tissue cultures or animal models. EIEC isolates may also be identified using PCR assays to test for the presence of *inv* genes. These assays, however, will detect both EIEC and *Shigella* spp., so additional assays are needed for differentiation.

6. Target Populations

All populations are susceptible to EIEC infections.

7. Food Analysis

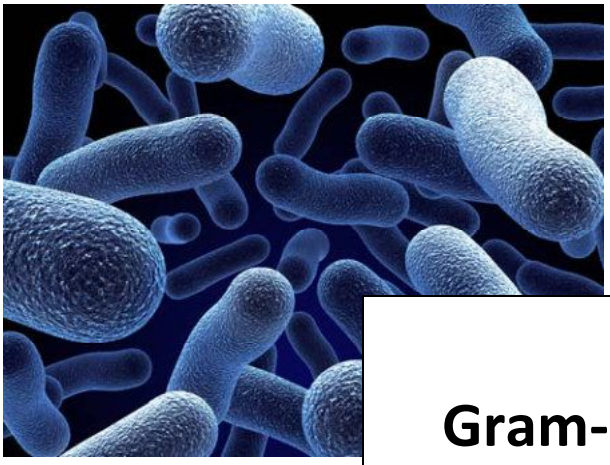
Presence of EIEC in foods can be determined by plating culture enrichment of food samples onto media that are selective and differential for *E. coli* and testing the isolates for the presence of *inv* genes. EIEC in foods can also be detected using *inv* gene-specific PCR assays, testing either directly or on food-sample enrichments.

8. Examples of Outbreaks

See Frequency section, above, and CDC's Morbidity and Mortality Weekly Reports**.**

9. Resources

- Loci index for genome
- GenBank Taxonomy Database



Gram-Positive Bacteria

Bad Bug Book

Foodborne Pathogenic Microorganisms and Natural Toxins

Clostridium perfringens

1. Organism

Clostridium perfringens is an anaerobic (but aerotolerant) Gram-positive, spore-forming rod that produces enterotoxin. The bacterium is relatively cold-tolerant, and its spores are heat-resistant. Non-pathogenic *C. perfringens* is widely distributed in the environment and is frequently found in the intestines of humans and many domestic and feral animals. Spores of the organism persist in soil, sediments, and areas subject to human or animal fecal pollution.

Among the many isotypes of *C. perfringens*, type A almost always contains the *cpe* gene (the enterotoxin gene, which causes food poisoning), and types B, C, D, and E sometimes contain this gene.

2. Disease

Foodborne illness caused by *C. perfringens* can take two forms.

1) The ***gastroenteritis form*** is very common and often is mild and self-limiting. Depending on the strain, it may also develop as more severe gastroenteritis that leads to damage of the small intestine and, potentially, but rarely, fatality.
2) The other form, ***enteritis necroticans or "pig-bel disease"*** (a name reportedly derived from pidgin English, referring to the characteristic swollen bellies and other severe symptoms that resulted from feasts on contaminated pork in New Guinea), is rare in the United States, more severe than the other form of the illness, and often fatal.

For Consumers: A Snapshot

Once this bacterium (estimated to be the second leading bacterial cause of foodborne illness in the U.S.) is eaten in contaminated food, it makes a toxin in the intestines. The toxin causes two major kinds of foodborne illness (and can cause other diseases transmitted in ways other than food). (1) One of the illnesses is very common, and the usually mild cramps and watery diarrhea start within 8 to 16 hours. For most people, symptoms go away by themselves in 24 hours, although they can be worse and last up to a week or two in very young or old people, or longer in people with weak immune systems (for example, people with HIV/AIDS or people on cancer chemotherapy or drugs that treat rheumatoid arthritis by lowering the actions of the immune system). The more serious, longer-lasting cases, especially, should be treated to prevent complications, like fluid imbalance that can cause heart-rhythm problems and other problems. (2) The other illness, called "pig-bel" (enteritis necroticans), is much more severe and often fatal, but is very rare in the U.S. Symptoms include pain and gassy bloating in the abdomen, diarrhea (maybe bloody), and vomiting. Knowing more about the bacterium, *Clostridium perfringens*, can help you understand how to protect yourself. The bacteria make tiny spores – a survival mode in which they make an inactive form that can exist without nutrition and that develops very tough protection against the outside world – which can survive cooking. After food is cooked, the spores can turn into full-fledged bacteria as the food cools – and here's the most important part: these bacteria multiply much faster than do most other kinds of bacteria. That means that if you cook meats (one of the higher-risk foods for this "bug") or other foods, then leave them at room temperature, this bacterium can multiply to levels that can make you sick a lot faster than other bacteria can. Refrigerating food within a couple of hours of cooking, or sooner, slows down the bacteria and greatly lowers your chance of illness. And remember that this bacterium also can contaminate raw foods, like vegetables. Washing your fresh produce in clean, running water helps protect you.

Both forms of the disease result from ingestion of large numbers of *C. perfringens*, which replicates much more quickly than do most other bacteria. This raises the likelihood that, compared with other bacteria, *C. perfringens* will more quickly reach pathogenic levels in contaminated food left unrefrigerated and that consumers who eat the food may ingest large doses of the bacterium.

- **Mortality**: In 1999, the Centers for Disease Control and Prevention (CDC) estimated that, overall, *C. perfringens* annually accounts for 26 deaths in the U.S.

- ***Common gastroenteritis form***: A few deaths resulting from diarrhea-induced dehydration and other complications have been reported, and usually were among debilitated or elderly people.

- ***Pig-bel form (enteritis necroticans)***: This disease is often fatal. As noted, it is extremely rare in the U.S.

- **Infective dose:** Symptoms are caused by ingestion of large numbers ($> 10^6$) vegetative cells or $>10^6$ spores/g of food. Toxin production in the digestive tract (or *in vitro*) is associated with sporulation. This disease is characterized as a food infection; only one episode has ever implied the possibility of intoxication (i.e., disease from preformed toxin).

- **Onset**: Symptoms occur about 16 hours after consumption of foods containing large numbers ($>10^6$ live vegetative cells or $>10^6$ spores) of *C. perfringens* capable of producing the enterotoxin.

- **Illness / complications:** Complications are rare in the typical, mild gastroenteritis form of the disease, particularly among people under 30 years old. Elderly people are more likely to have prolonged or severe symptoms, as are immunocompromised people. The more severe form of the disease may cause necrosis of the small intestine, peritonitis, and septicemia.

- **Symptoms**:

  Gastroenteritis form: Common characteristics include watery diarrhea and mild abdominal cramps.

  Pig-bel form (enteritis necroticans): Abdominal pain and distention, diarrhea (sometimes bloody), vomiting, and patchy necrosis of the small intestine.

- **Duration**: The milder form of the disease generally lasts 12 to 24 hours. In the elderly or infants, symptoms may last 1 to 2 weeks.

- **Route of entry**: Oral.

- **Pathway**: CPE protein usually is released into the intestines when the vegetative cells lyse on completion of sporulation. This enterotoxin is responsible for the clinical presentation in humans. The enterotoxin induces fluid and electrolyte losses from the GI tract. The principal target organ for CPE is believed to be the small intestine.

Pig-bel disease involves production of beta toxin, which is highly trypsin-sensitive. Of note: consumption of large amounts of sweet potatoes, which generally contain trypsin inhibitor, could contribute to progression of the disease. The effects of low gastrointestinal levels of trypsin appear to have been demonstrated in Germany around the end of World War II and post-war, when starvation and high levels of potato consumption contributed to low levels of this enzyme in the population. These were thought to have been major cofactors in the occurrence of pig-bel disease in Germany during that period.

3. Frequency

Perfringens poisoning is one of the most commonly reported foodborne illnesses in the U.S. The CDC estimates that 965,958 domestically acquired cases occur annually in the U.S., second only to *Salmonella* when considering bacterial causes of foodborne illness. Thirty-four outbreaks in 2006 (i.e., not including isolated cases) included 1,880 cases. At least 51 outbreaks were reported annually in the U.S. from 2001 to 2005. Typically, 50 to 100 people are affected in one outbreak. It is probable that many outbreaks go unreported, because the implicated foods and patients' feces are not tested routinely for *C. perfringens* or its toxin.

4. Food Sources / potentiating characteristics of the organism

In most instances, the actual cause of poisoning by this organism is temperature abuse of cooked foods. Small numbers of the organism often are present after the food is cooked, due to germination of its spores, which can survive high heat and can multiply rapidly as a result of a fast doubling time (<10 minutes for vegetative cells), depending on temperature and food matrix. Therefore, during cool-down (109-113°F) and storage of prepared foods, this organism can reach levels that cause food poisoning much more quickly than can other bacteria.

Meats (especially beef and poultry), meat-containing products (e.g., gravies and stews), and Mexican foods are important vehicles for *C. perfringens* foodborne illness, although it is also found on vegetable products, including spices and herbs, and in raw and processed foods. Spores of some *C. perfringens* strains can survive boiling water for an hour or longer in a relatively protective medium (e.g., a cooked-meat medium).

5. Diagnosis

Perfringens poisoning is diagnosed by its symptoms and the typical delayed onset of illness. Diagnosis is confirmed by detection of the toxin in patients' feces. Bacteriologic confirmation can also be done by finding exceptionally large numbers of the bacteria in implicated foods or in patients' fecal samples.

6. Target populations

Institutional settings (such as school cafeterias, hospitals, nursing homes, prisons, etc.), where large quantities of food are prepared several hours before serving, are the most common circumstance in which *C. perfringens* poisoning occurs. The young and elderly are the most frequent victims of *C. perfringens* poisoning. As with other infections, immunocompromised people are at higher risk of severe illness than are others; e.g., those with HIV/AIDS or undergoing cancer chemotherapy or immunosuppressive drugs for rheumatoid arthritis or other inflammatory conditions.

7. Food Analysis

Standard bacteriological culturing procedures are used to detect the organism in implicated foods and in feces of patients. Serological assays are used for detecting enterotoxin in the feces of patients and for testing the ability of strains to produce toxin. With the introduction of PCR-based methods, toxin typing using antiserum neutralization tests in mice is no longer practical.

8. Examples of outbreaks

For more information about outbreaks, see CDC's Morbidity and Mortality Weekly Reports**.**

9. Other Resources:

Loci index for genome *Clostridium perfringens* from GenBank

Bad Bug Book

Foodborne Pathogenic Microorganisms and Natural Toxins

Staphylococcus aureus

1. Organism

Staphylococcal species are Gram-positive, non-motile, catalase-positive, small, spherical bacteria (cocci), which, on microscopic examination, appear in pairs, short chains, or bunched in grape-like clusters. Staphylococci are ubiquitous and impossible to eradicate from the environment. Many of the 32 species and subspecies in the genus *Staphylococcus* are potentially found in foods due to environmental, human, and animal contamination.

Several staphylococcal species, including both coagulase-negative and coagulase-positive strains, have the ability to produce highly heat-stable enterotoxins that cause gastroenteritis in humans. *S. aureus* is the etiologic agent predominantly associated with staphylococcal food poisoning.

S. aureus is a versatile human pathogen capable of causing staphylococcal food poisoning, toxic shock syndrome, pneumonia, postoperative wound infection, and nosocomial bacteremia. *S. aureus* produces a variety of extracellular products, many of which act as virulence factors. Staphylococcal enterotoxins can act as superantigens capable of stimulating an elevated percentage of T-cells.

S. aureus is one of the most resistant non-spore-forming human pathogens and can survive for extended periods in a dry state. Staphylococci are mesophilic. *S. aureus* growth, in general, ranges from 7°C to 47.8°C, with 35°C being the optimum temperature for growth. The growth pH range is between 4.5 and 9.3, with an optimum between 7.0 and 7.5. Staphylococci are atypical, in that they are able to grow at low levels of water activity, with growth demonstrated at a_w as low as 0.83, under ideal conditions. Optimum growth of *S. aureus* occurs at a_w of >0.99. For the most part, strains of *S. aureus* are highly tolerant to salts and sugars.

For Consumers: A Snapshot

This bacterium, often called "Staph" for short, can cause food poisoning. It's very common in the environment and can be found in soil, water, and air, and on everyday objects and surfaces. It can live in humans and animals. *Staphylococcus aureus* is found in foods and can make toxins (enterotoxins) that might not be destroyed by cooking, although the bacterium itself can be destroyed by heat. These toxins can cause nausea, stomach cramps, vomiting, and diarrhea. In more severe cases, the toxins may cause loss of body fluid (dehydration), headache, muscle cramps, and temporary changes in blood pressure and heart rate. The illness usually is intense, but normally lasts from just a few hours to a day. The toxins are fast-acting; they cause symptoms within 1 to 7 hours after contaminated food is eaten. Follow basic food-safety tips to help protect yourself from this illness. Outbreaks often have been linked to foods that require a lot of handling when they're being processed or prepared and/or weren't kept at proper refrigerator temperature (40°F or below). To help protect yourself, it's especially important to wash your hands well when handling food, properly clean your cooking equipment and surfaces, keep your cooked foods from touching raw foods or unclean equipment or surfaces, and keep foods refrigerated at 40°F or below. Examples of foods that have been linked to this type of food poisoning include meat and meat products; poultry and egg products; salads, such as egg, tuna, chicken, potato, and macaroni; bakery products, such as cream-filled pastries, cream pies, and chocolate éclairs; sandwich fillings; and milk and dairy products.

Staphylococcal Enterotoxins (SE)

Staphylococcal enterotoxins are single-chain proteins with molecular weights of 26,000 to 29,000. They are resistant to proteolytic enzymes, such as trypsin and pepsin, which allows them to transit intact through the digestive tract. There are five classical enterotoxin serotypes: SEA, SEB, SEC1,2,3, SED, and SEE and the more recently described SEG, SEH, and SEI; all exhibit emetic activity. There are also SE-like enterotoxin serotypes, SElJ-SElU; these SE-like designations have not been confirmed to exhibit emetic activity. The different SE serotypes are similar in composition and biological activity, but are different in antigenicity and are identified serologically as separate proteins.

2. Illness

Staphylococcal food poisoning (staphyloenterotoxicosis; staphyloenterotoxemia) is the name of the condition caused by the enterotoxins. Treatment typically involves managing the complications.

- **Mortality**: Death from staphylococcal food poisoning is uncommon, although it has occurred among the elderly, infants, and severely debilitated people.

- **Infective dose:** The intoxication dose of SE is less than 1.0 microgram. This toxin level is reached when *S. aureus* populations exceed 100,000 organisms/g in food. This level is indicative of unsanitary conditions in which the product can be rendered injurious to health. In highly sensitive people, ingestion of 100 to 200 ng of enterotoxin can cause symptoms. The population of *S. aureus* at the time of analysis may be significantly different, and not representative of, the highest population that occurred in the product. This should be taken into consideration when examining foods.

- **Onset**: The onset of symptoms usually is rapid (1 to 7 hours) and in many cases acute, depending on individual susceptibility to the toxin, amount of toxin ingested, and general health.

- **Illness / complications:** Staphylococcal food poisoning generally causes self-limiting, acutely intense illness in most people. Not all people demonstrate all symptoms associated with the illness. The most common complication is dehydration caused by diarrhea and vomiting.

- **Symptoms**: When ingested, the enterotoxin may rapidly produce symptoms, which commonly include nausea, abdominal cramping, vomiting, and diarrhea. In more severe cases, dehydration, headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur.

- **Duration**: The illness is relatively mild and usually lasts from only a few hours to one day; however, in some instances, the illness is severe enough to require hospitalization.

- **Route of entry**: Consumption of food contaminated with enterotoxigenic *S. aureus* or ingestion of the preformed enterotoxin.

- **Pathway**: Staphylococcal enterotoxins are stable in the gastrointestinal tract and indirectly stimulate the emetic reflex center by way of undetermined molecular events. It

is thought that the vagus nerve is involved in the sequence of events that produce the emetic response.

3. Frequency

S. aureus is the cause of sporadic food poisoning episodes around the world. The Centers for Disease Control and Prevention (CDC) estimates that, in the United States, staphylococcal food poisoning causes approximately 241,188 illnesses, 1,064 hospitalizations, and 6 deaths each year. The true incidence is unknown for a number of reasons, including poor responses from victims during interviews with health officials; misdiagnosis of the illness, which may be symptomatically similar to other types of food poisoning (such as vomiting caused by *Bacillus cereus* emetic toxin); inadequate collection of samples for laboratory analyses; improper laboratory examination; and, most important, many victims do not seek medical attention because of the short duration of the illness. Although it is under-reported, staphylococcal food poisoning remains a common cause of foodborne illness as indicated by the recent Centers for Disease Control and Prevention (CDC) report (see Resources section, Scallan *et al*.).

4. Sources

Staphylococci are widely distributed in the environment. They can be found in the air, dust, sewage, water, milk, and food, or on food equipment, environmental surfaces, humans, and animals.

Foods frequently implicated in staphylococcal food poisoning include meat and meat products; poultry and egg products; salads, such as egg, tuna, chicken, potato, and macaroni; bakery products, such as cream-filled pastries, cream pies, and chocolate éclairs; sandwich fillings; and milk and dairy products. Foods that require considerable handling during preparation and are kept slightly above proper refrigeration temperatures for an extended period after preparation are frequently involved in staphylococcal food poisoning.

Unless heat processes are applied, staphylococci are expected to exist in any and all foods that are handled directly by humans or are of animal origin. Destruction of viable cells by heat does not destroy the biological activity of preformed staphylococcal enterotoxins. These toxins are highly heat stable and can remain biologically active.

Staphylococci are present in the nasal passages and throats and on the hair and skin of 50% or more of healthy individuals. The incidence is even higher among those who associate with sick people and hospital environments. Contamination may be introduced into foods by direct contact with workers with hand or arm lesions caused by *S. aureus*, or by coughing and sneezing, which is common during respiratory infections. Food handlers are frequently the source of food contamination in staphylococcal outbreaks; however, equipment and environmental surfaces also can be sources.

Avoiding time and temperature abuse of food products that are at high risk of containing *S. aureus* is essential in preventing the proliferation of the bacterium and subsequent production of enterotoxin. In cases of human intoxication, the implicated food usually has not been kept at a refrigerated temperature of <10°C or has not been kept hot enough (>45°C).

5. Diagnosis

Staphylococcal food poisoning is diagnosed based on isolation of the pre-formed enterotoxin or the isolation of enterotoxigenic staphylococci from the suspect food consumed and/or the vomitus or feces of the patient. The food history and rapid onset of symptoms often are sufficient to diagnose this type of food poisoning. Suspect foods are collected and examined for presence of viable staphylococci and preformed enterotoxin. The most conclusive test is the linking of an illness with a specific food, or in cases in which multiple vehicles exist, detection of pre-formed enterotoxin in food sample(s).

6. Target populations

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

7. Food Analysis

A number of serological methods have been developed for detection of pre-formed enterotoxin in foods. These same methods are also utilized for determining the enterotoxigenicity of *S. aureus* isolate from a food product.

Enrichment isolation and direct plating are the methods frequently used for detecting and enumerating *S. aureus* in foods. Non-selective enrichment is useful for demonstrating presence of injured cells, whose growth is inhibited by selective enrichment media. Enumeration by enrichment isolation, or selective enrichment isolation, may be achieved by determining either the direct plate count or the most probable number (MPN) of *S. aureus* present. The MPN procedure is recommended for surveillance of products expected to have a small population of *S. aureus* and a large population of competing organisms. Direct plating method is suitable for analysis of foods in which a population of *S. aureus* is expected to be greater than 100 CFU/g.

During outbreak investigations, it is recommended that foods be tested for pre-formed enterotoxin and to determine enterotoxigenicity of isolates. Currently ELISA-based methods are those most widely used to identify staphylococcal enterotoxins. Several commercially available enzyme-linked immunosorbent assays use both monoclonal and polyclonal antibodies. The intensity of the color reaction or florescence is proportional to the amount of toxin present in the sample. These extraction and detection methods are described in detail in the BAM online chapter 13A.

Steps during food processing and preservation, including treatment with heat, acidulation, or chemicals, and other treatments stress the staphylococcal enterotoxin protein. A processed product may have serologically inactive and undetectable toxin, while the toxin protein remains biologically active and can cause illness. Procedures have been developed to chemically treat suspect samples that may contain denatured enterotoxins, to restore serological activity, so that the toxin can be detected using classical serological methods.

When food has been treated to eliminate viable microorganisms, as in pasteurization or heating, DNA-based techniques, such as PCR, or direct microscopic observation of the food (if the cells were not lysed), can assist in identification and diagnosis. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) are the most common molecular subtyping techniques used for staphylococcal species; these are powerful tools that can be used when viable

staphylococci are isolated from the implicated food, victims, and suspected carriers, such as food handlers.

8. Examples of Outbreaks

Example of a typical outbreak:

Among 5,824 children who had eaten lunch served in 16 elementary schools in Texas, 1,364 became ill. The lunches were prepared in a central kitchen and transported to the schools by truck. Epidemiologic studies revealed that 95% of the ill children had eaten chicken salad. The day before, frozen chickens had been boiled for 3 hours, then deboned, cooled to room temperature with a fan, ground into small pieces, placed into l2-inch-deep aluminum pans, and stored overnight in a walk-in refrigerator, at 42°F to 45°F.

The next morning, the remaining ingredients were added and blended in with an electric mixer. The food was placed in thermal containers and transported to the various schools, from 9:30 a.m. to 10:30 a.m., where it was kept at room temperature until served, from 11:30 a.m. to noon. Bacteriologic examination of the chicken salad revealed large numbers of *S. aureus*.

Example of outbreak that is not typical:

In 1989, multiple staphylococcal foodborne diseases were associated with canned mushrooms. Enterotoxin type A (SEA) was identified in several samples of unopened cans from the same lot. (CDC Morbidity and Mortality Weekly Report, June 23, 1989, Vol. 38, #24.)

S. intermedius, typically considered a veterinary pathogen, was isolated from butter blend and margarine implicated in a 1991 food poisoning outbreak. SEA was detected in both clinical and food isolates implicated in this food-related outbreak involving more than 265 cases in the western US. (Khambaty *et al.*, 1994)

Recent outbreaks:

For more information about recent outbreaks, see CDC's Morbidity and Mortality Weekly Reports.

9. Resources

A Loci index for genome *Staphylococcus aureus* is available from GenBank.

Bennett RW. 2005. Staphylococcal Enterotoxin and Its Rapid Identification in Foods by Enzyme-Linked Immunosorbant Assay-Based Methodology. *J. of Food Protection* 68: 1264-1270.

Khambaty FM, Bennett RW, Shah DB. 1994. Application of pulsed-field gel electrophoresis to the epidemiological characterization of *Staphylococcus intermedius* implicated in food related outbreak. Epidermiol. Infect. 113:75-81.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, *et al.* 2011. Foodborne Illness Acquired in the United States – Major Pathogens. Emerg Infect Dis 17(1):7-15.

Seo KS, Bohach GA. 2007. *Staphylococcus* aureus in Doyle MP and Beuchat LR, *Food Microbiology*, 3rd ed. ASM Press, Washington D.C. pp. 493-518.

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Bacillus cereus and other *Bacillus* species

1. Organism

Bacillus cereus is a Gram-positive, facultatively anaerobic, endospore-forming, large rod. These and other characteristics, including biochemical tests, are used to differentiate and confirm the presence of *B. cereus*, although these characteristics are shared with *B. mycoides*, *B. pseudomycoides*, *B. thuringiensis* and *B. anthracis*. Differentiation of these organisms depends on:

- determination of motility (most *B. cereus* strains are motile)
- presence of toxin crystals (*B. thuringiensis*)
- hemolytic activity (*B. cereus* and others are beta hemolytic, whereas *B. anthracis* usually is non-hemolytic)
- rhizoid growth, which is characteristic of *B. cereus* var. mycoides.

B. weihenstephanensis, also a member of this group, is a psychrotrophic strain, and thus can grow at refrigerated temperatures.

Production of the enterotoxin associated with the vomiting form of *B. cereus* food poisoning (cereulide, described below) has been detected in other bacilli, including *B. weihenstephanensis*. This suggests that the plasmid carrying the emetic toxin can undergo lateral transfer, conferring the same properties to otherwise non-pathogenic strains.

B. cereus is widespread in the environment and often is isolated from soil and vegetation. The optimal growth temperature is 28°C to 35°C, with a

For Consumers: A Snapshot

Bacillus cereus might cause many more cases of foodborne illness than is known. One reason it's under-reported may be that most people have fairly mild, brief symptoms, so they don't seek medical attention. But it can cause serious illness in some people, as described below. Often called "*B. cereus*," this bacterium can cause two different types of sickness. (1) In the first type, after contaminated food is eaten the bacteria make a toxic substance in the small intestine. This can lead to diarrhea, cramps, and, sometimes, nausea (but usually not vomiting). Many kinds of contaminated foods have been linked to this illness. Symptoms start in about 6 to 15 hours and usually clear up within a day or so. (2) The second type occurs if *B. cereus* makes a different kind of toxin in contaminated food. It most often affects rice and other starchy foods. It causes nausea and vomiting in a half-hour to 6 hours and usually clears up in about a day. Both kinds of illness generally go away by themselves, but can cause serious complications, although rarely in otherwise healthy people. As with all infections, people who have weak immune systems (because they have certain other diseases or take medications that weaken the immune system) are much more likely to suffer serious consequences. One of the most important things you can do to protect yourself from infection with *B. cereus* is to keep your food refrigerated at 40°F or lower. The reason is that, at higher temperatures, *B. cereus* can form spores – a survival mode in which they make an inactive form that can exist without nutrition and that develops very tough protection against the outside world – that grow and turn into more *B. cereus* bacteria. The more bacteria, the more toxin, and the greater the chance that you'll get sick. Cooking may kill the bacteria, but it might not disable the toxin that causes the vomiting type of illness. And don't stop at refrigeration, because a related *Bacillus* bacterium can survive and grow at refrigerator temperature. Add other food-safety measures – good hygiene, like washing your hands, foods and utensils, and cooking setting; and keep raw and cooked foods separate.

minimum growth temperature of 4°C and a maximum of 48°C. Growth can occur in pH ranges from 4.9 to 9.3, and the organism tolerates 7.5% salt concentration.

2. Disease

B. cereus food poisoning is the general description of illness associated with this organism, although two recognized types of illness are caused by two distinct metabolites (toxins):

The ***diarrheal type*** of illness is caused by a large-molecular-weight protein.

The ***vomiting (emetic) type*** of illness is associated with cereulide, an ionophoric low-molecular-weight dodecadepsipeptide that is pH-stable and heat- and protease- resistant. The non-antigenic peptide is stable after heating at 121°C for 30 minutes, cooling at 4°C for 60 days, and at a pH range of 2 to 11.

- **Mortality**: Albeit rare, the emetic enterotoxin of *B. cereus* foodborne illness has been implicated in liver failure and death in otherwise healthy individuals. Similarly, a newly identified cytotoxin has been isolated from a *B. cereus* strain that caused a severe outbreak and three deaths.

- **Infective dose:** The presence of large numbers of *B. cereus* (greater than 10^6 organisms/g) in a food is indicative of active growth and proliferation of the organism and is consistent with a potential human health hazard. The number of organisms most often associated with human illness is 10^5 to 10^8; however, the pathogenicity arises from preformed toxin.

- **Onset**:

  Diarrheal type: 6 to 15 hours after consumption of contaminated food.

  Emetic type: 0.5 to 6 hours after consumption of contaminated foods.

- **Disease / complications:** Although both forms of foodborne illness associated with the diarrheal and vomiting toxins produced by *B. cereus* are generally mild and self-limiting, more severe and fatal forms of the illness have been reported. Other clinical manifestations of *B. cereus* invasion and infection that have been observed include severe systemic and pyogenic infections, gangrene, septic meningitis, cellulitis, panophthalmitis, lung abscesses, infant death, and endocarditis, and, in cows, bovine mastitis.

- **Symptoms**:

  Diarrheal type: The symptoms of *B. cereus* diarrheal-type food poisoning include watery diarrhea, abdominal cramps, and pain, mimicking those of *Clostridium perfringens* food poisoning. Nausea may accompany diarrhea, but vomiting (emesis) rarely occurs.

  Emetic type: The symptoms of the emetic type of food poisoning include nausea and vomiting, paralleling those caused by *Staphylococcus aureus* foodborne intoxication.

- **Duration of symptoms**: The symptoms usually subside after 24 hours of onset.

- **Route of entry**: Consumption of food contaminated with enterotoxigenic *B. cereus* or with the emetic toxin.

- **Pathway**: Cereulide has been shown to be toxic to mitochondria by acting as a potassium ionophore. Using a house musk shrew animal model, researchers have found that a serotonin5-HT_3 receptor-mediated mechanism is associated with the emetic syndrome. Two of the diarrheal enterotoxins are composed of multicomponent proteins that have dermonecrotic and vascular permeability activities and cause fluid accumulation in ligated rabbit ileal loops. The third type of enterotoxin is a member of the β-barrel toxin family and is similar to the β-toxin of *Clostridium perfringens.*

3. Frequency

In a recent Centers for Disease Control and Prevention (CDC) report on domestically acquired foodborne illness in the United States, the estimated number of episodes of *B. cereus* illness annually was given as 63,400. The numbers of confirmed outbreaks reported to the CDC in 2005, 2006, and 2007 were 4, 3, and 6 and affected 69, 35, and 100 people, respectively. However, an average of 37.6 suspected outbreaks occurred during this same period, affecting more than 1,000 people. Foods that were associated with outbreaks included beef, turkey, rice, beans, and vegetables. Other outbreaks may go unreported or are misdiagnosed because of symptomatic similarities to *Staphylococcus aureus* intoxication (*B. cereus* vomiting type) or *Clostridium perfringens* food poisoning (*B. cereus* diarrheal type).

4. Sources

A wide variety of foods, including meats, milk, vegetables, and fish, have been associated with the diarrheal-type food poisoning. The vomiting-type outbreaks generally have been associated with rice products; however, other starchy foods, such as potato, pasta, and cheese products, also have been implicated. Food mixtures, such as sauces, puddings, soups, casseroles, pastries, and salads, frequently have been linked with food-poisoning outbreaks.

5. Diagnosis

Confirmation of *B. cereus* as the etiologic agent in a foodborne outbreak requires either (1) isolation of strains of the same serotype from the suspect food and feces or vomitus of the patient, (2) isolation of large numbers of a *B. cereus* serotype known to cause foodborne illness from the suspect food or from the feces or vomitus of the patient, or (3) isolation of *B. cereus* from suspect foods and determination of their enterotoxigenicity by serological (diarrheal toxin) or biological (diarrheal and emetic) tests. The rapid-onset time to symptoms in the emetic form of the disease, coupled with some food evidence, is often sufficient to diagnose this type of food poisoning.

6. Target Populations

All people are believed to be susceptible to *B. cereus* food poisoning.

7. Food Analysis

A variety of methods have been recommended for the recovery, enumeration, and confirmation of *B. cereus* in foods. More recently, a serological method has been developed for detecting the

putative enterotoxin of *B. cereus* (diarrheal type) isolates from suspect food sources. Recent investigations suggest that the vomiting-type toxin can be detected through animal models (cats, monkeys) or, possibly, by cell culture.

8. Examples of Outbreaks

CDC OutbreakNet Foodborne Outbreak Online Database

9. Other Resources

- Loci index for genome *Bacillus cereus*
- GenBank Taxonomy database
- "Produce Handling and Processing Practices" (1997) Emerging Infectious Diseases 3(4).
- Surveillance for Foodborne Disease Outbreaks – U.S., 2006

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Streptococcus species

1. Organism

Streptococcus A is not a major cause of foodborne illness, although serious complications occasionally develop if foodborne illness does occur. Streptococci can be found on the skin; the mucous membranes of the mouth, respiratory, alimentary, and genitourinary tracts of human and animals; and in some plants, soil, and bodies of dirty water. They are opportunistic pathogens. Optimum incubation temperature is usually 37°C, with relatively wide variations among species.

(Illnesses caused by *Streptococcus* A that are not typically transmitted by food, such as those arising from skin infections – e.g., necrotizing fasciitis and streptococcal toxic shock syndrome – will not be addressed in this chapter.)

The genus *Streptococcus* is comprised of Gram-positive, catalase-negative, microaerophilic cocci that are nonmotile and occur in chains or pairs, and in long chains in broth culture. Cells are normally spherical, ovoid, and less than 2 µm in diameter.

The type species is *Streptococcus pyogenes* Rosenbach 1884, 23. The genus is defined by a combination of antigenic, hemolytic, and physiologic characteristics that are further refined into Groups A, B, C, D, E, F, G, N, etc.

For Consumers: A Snapshot

You've probably heard of "Strep throat," but might not know that contaminated food is one way you can be infected with *Streptococcus*, the bacterium that causes it. *Streptococcus* isn't a leading cause of illness from food, but the illness that it does cause can develop into more serious problems. Seeing a health professional, to get treatment, is important. Some people infected with foodborne *Streptococcus* have no symptoms, but those who do will start to have them in about 1 to 3 days after eating contaminated food. They may start with red, sore throat (with or without white patches), painful swallowing, high fever, nausea, vomiting, headache, discomfort, and runny nose. The symptoms usually go away in about 4 days. However, 2 or 3 weeks afterwards, some people develop scarlet fever, which includes a rash, or rheumatic fever, which can harm the heart and other parts of the body, or *Streptococcus* could spread to other organs and cause serious illness or death. Children 5 to 15 years old and people with weak immune systems are more likely than others to develop the serious forms of the illness. Infected food handlers are thought to be the main way food is contaminated with *Streptococcus*. In most cases, the food was left at room temperature for too long, letting the bacteria multiply to harmful levels. Keeping food refrigerated at 40°F or below is one way you can protect yourself

Groups A and D can be transmitted to humans via food. This chapter will focus on Group A, since most group D species have been reclassified as enterococci and are covered in a separate chapter. The most important species in Group A is *S. pyogenes*.

2. Disease

- **Mortality**: In otherwise healthy people, most cases of foodborne *Streptococcus* infection are relatively mild. In patients who develop invasive disease (most likely to occur in people with underlying health issues, such as those who are immunocompromised), the death rate is estimated at 13%.

- **Infective dose**: The infectious dose for group A *Streptococcus* probably is fewer than 1,000 organisms.

- **Onset**: Usually 1 to 3 days. If rheumatic fever or scarlet fever develop, they usually do so 2 to 3 weeks after the initial infection (e.g., sore throat).

- **Disease / complications:** Some foodborne *Streptococcus* Group A infections are asymptomatic. Of those that are symptomatic, most manifest as pharyngitis (and are commonly referred to as "Strep throat"). Although they may be painful and uncomfortable, they usually are relatively mild. However, the infection may also result in complications, such as tonsillitis, scarlet fever, rheumatic fever, and septicemic infections.

- **Symptoms**: Sore, inflamed throat, on which white patches may or may not appear; pain on swallowing; high fever; headache; nausea; vomiting; malaise; and rhinorrhea. In cases of scarlet fever, a rash may develop, which begins on the sides of the chest and abdomen and may spread. Symptoms of rheumatic fever, which affects collagen and causes inflammation, appear in the heart, joints, skin, and/or brain.

- **Duration of symptoms**: Symptoms of uncomplicated illness generally begin to resolve within about 4 days.

  Antimicrobials, such as penicillin (or ceftriaxone), azithromycin, and clindamycin, are used to treat Group A *Streptococcus* disease. Other alternatives for prevention and control of the disease that are being considered include vaccines and phage and immunologic therapies.

- **Route of entry**: Oral (although the organism also may be spread through vehicles other than food and causes other types of serious illness that are not addressed in this chapter).

- **Pathway**: Group A *Streptococcus* putatively is associated with many virulence factors, such as streptolysin O, streptolysin S, erythrogenic toxin, pyrogenic toxin, streptokinase, superantigens, protein SIC, SpeB, M-protein family, fibronectin-binding proteins, C5a peptidase, etc. The mechanisms and functionality of these virulence factors are extremely complicated and not very well defined. However, it is clear that some surface proteins contribute to adherence to cells and, possibly, the bacterium's internalization in cells and to colonization.

3. Frequency

A 2011 report by the Centers for Disease Control and Prevention (CDC) estimated that 11,217 cases of foodborne Streptococcal illness occur annually in the United States.

4. Sources

Food handlers are thought to be a major source of food contamination with *Streptococcus* Group A. Foods that have been associated with *Streptococcus* A contamination include milk (both pasteurized and unpasteurized), ice cream, cream, eggs, cooked seafood, ground ham, potato salad, egg salad, custard, rice pudding, and shrimp salad. In almost all cases, the foods were allowed to stand at room temperature for several hours between the time of preparation and the time of consumption.

5. Diagnosis

Culturing of nasal and throat swabs, sputum, blood, suspect food, and environmental samples.

6. Target Populations

All people are susceptible. However, children, immunocompromised people, and people 65 years or older, in nursing homes, are more vulnerable. Scarlet fever and rheumatic fever are more common among children 5 to 15 years old than among adults.

7. Food Analysis

The suspect food is examined microbiologically by nonselective and selective medium techniques, which can take up to 7 days. Group specificities are determined by Lancefield group-specific antisera. Both biochemical and DNA techniques are used for identification.

8. Examples of Outbreaks

For more information about recent outbreaks, see CDC's Morbidity & Mortality Weekly Report.

9. Other Resources

- Loci index for genome Streptococcus
- Taxonomy database
- FAQ's about Group A *Streptococcus* from the CDC. (Note: not all of the information at this site relates to *Streptococcus* transmitted via food.)

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Listeria monocytogenes

1. Organism

Listeria monocytogenes is a Gram-positive, rod-shaped, facultative bacterium, motile by means of flagella, that is among the leading causes of death from foodborne illness. It has 13 serotypes, including 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7. Among them, serotypes 1/2a, 1/2b, and 4b have been associated with the vast majority of foodborne infections.

L. monocytogenes is hardy; it is salt-tolerant and not only can survive in temperatures below 1°C, but also grow in these conditions, unlike many other pathogens. It is also notable for its persistence in food-manufacturing environments. The bacterium is ubiquitous in the environment and can be found in moist environments, soil, and decaying vegetation.

Of the five other species in the genus *Listeria* – *L. grayi, L. innocua. L. ivanovii, L. seeligeri* and *L. welshimeri* – only *L. ivanovii* is considered pathogenic, and mainly in ruminants, rather than in humans.

For Consumers: A Snapshot

Although the number of people infected by foodborne *Listeria* is comparatively small, this bacterium is one of the leading causes of death from foodborne illness. It can cause two forms of disease. One can range from mild to intense symptoms of nausea, vomiting, aches, fever, and, sometimes, diarrhea, and usually goes away by itself. The other, more deadly form occurs when the infection spreads through the bloodstream to the nervous system (including the brain), resulting in meningitis and other potentially fatal problems. Pregnant women are more susceptible to *Listeria* infection than are most other people, and although they generally recover, their babies usually don't survive. People with weak immune systems also are more vulnerable (for example, those with AIDS or chronic diseases, or who are on certain immune-suppressing arthritis drugs or cancer chemotherapy). Because our immune systems weaken as we age, the elderly also are especially vulnerable to this pathogen. *Listeria* cases have been traced back to several foods; for example, raw or under-pasteurized milk; smoked fish and other seafood; meats, including deli meats; cheeses (especially soft cheeses); and raw vegetables. *Listeria* is hardy; it tolerates salty environments and cold temperatures, unlike many other foodborne bacteria. You can help protect yourself from infection with *Listeria* by ***not*** drinking ***un***pasteurized milk (also called "raw" milk) or certain cheeses or other foods made with raw milk; and by cooking food according to instructions; washing fruits and vegetables; keeping raw foods from touching other foods, dinnerware, kitchen counters, etc.; and washing your hands and other things that have come into contact with raw foods.

2. Disease

- **Mortality**: Although not a leading cause of foodborne illness, *L. monocytogenes* is among the leading causes of death from foodborne illness. A recent report by the Centers for Disease Control and Prevention (CDC) estimated that domestically acquired foodborne *L. monocytogenes* causes 255 deaths in the U.S. annually. The severe form of the infection has a case-fatality rate of 15% to 30%, overall. When listerial meningitis occurs, the case-fatality rate may be as high as 70%; from septicemia, 50%, overall; and in perinatal/neonatal infections, more than 80%.

- **Infective dose**: The infective dose of *L. monocytogenes* is undetermined, but is believed to vary with the strain and susceptibility of the host, and the food matrix involved also may affect the dose-response relationship. In cases associated with raw or inadequately pasteurized milk, for example, it is likely that fewer than 1,000 cells may cause disease in susceptible individuals. As noted, however, the infective dose may vary widely and depends on a variety of factors.

- **Onset**: Gastroenteritis caused by *L. monocytogenes* has a relatively short incubation period, from a few hours to 2 or 3 days. The severe, invasive form of the illness can have a very long incubation period, estimated to vary from 3 days to 3 months.

- **Illness / complications:** *L. monocytogenes* infection causes two forms of disease in humans:

  1) non-invasive gastrointestinal illness, which generally resolves in otherwise healthy people.

  2) the much more serious, invasive form of the illness, which may cause septicemia and meningitis.

  Manifestations of *L. monocytogenes* infection tend to be host-dependent. In people with intact immune systems, it may cause acute febrile gastroenteritis, the less severe form of the disease. In vulnerable populations, however, the more severe form of the disease may result in sepsis and spread to the nervous system, potentially causing meningitis. In elderly and immunocompromised people who develop the severe form, it usually manifests in this manner.

  Pregnant women, who are disproportionately infected with *L. monocytogenes*, may experience mild, flu-like symptoms; however, their offspring do not fare as well – they may abort or be stillborn, and those born alive may have bacteremia and meningitis. One-third of confirmed cases of maternal-fetal *L. monocytogenes* infections lead to abortion or stillbirth.

- **Symptoms**: Otherwise healthy people might have mild symptoms or no symptoms if infected with *L. monocytogenes*, while others may develop fever, muscle aches, nausea and vomiting, and, sometimes, diarrhea. When the more severe form of the infection develops and spreads to the nervous system, symptoms may include headache, stiff neck, confusion, loss of balance, and convulsions.

- **Duration**: The duration of symptoms generally depends on the health status of the infected person. The symptoms can last from days to several weeks.

- **Route of entry**: Oral.

- **Pathway**: The pathogenesis of *L. monocytogenes* is unique, because the organism is able to spread directly from cell to cell in the host, rather than having to "travel" interstitially to reach other cells. Once the bacterium enters the host's monocytes, macrophages, or polymorphonuclear leukocytes, it can reproduce, and it is bloodborne. Groups of proteins on the *L. monocytogenes* cell surface enable it to survive in phagocytic cells and enhance its cell-to-cell spread.

3. Frequency

Based on a survey collected through 1997 by the Centers for Disease Control and Prevention (CDC), listeriosis was responsible for approximately 2,500 illnesses and 500 deaths in the United States annually. By 2008, the number of *L. monocytogenes* infections had declined 36 percent, compared to the period from 1996 to 1998. There was a moderate increase in the incidence of *L. monocytogenes* from 2008 to 2009; however, it was still lower than the incidence measured 10 years before that. More recently, the 2011 CDC report cited above estimated that *L. monocytogenes* causes 1,591 cases annually.

4. Sources

Many foods have been associated with *L. monocytogenes*. Examples include raw milk, inadequately pasteurized milk, chocolate milk, cheeses (particularly soft cheeses), ice cream, raw vegetables, raw poultry and meats (all types), fermented raw-meat sausages, hot dogs and deli meats, and raw and smoked fish and other seafood. *L. monocytogenes* can grow in refrigerated temperatures, which makes this organism a particular problem for the food industry.

Potential contamination sources include food workers, incoming air, raw materials, and food-processing environments. Among those, post-processing contamination at food-contact surfaces poses the greatest threat to product contamination.

5. Target Populations

The main target populations for listeriosis are:

- pregnant women/fetuses/neonates - perinatal and neonatal infections;
- persons immunocompromised by, for example, corticosteroids, anticancer drugs, graft suppression therapy, AIDS;
- cancer patients, particularly leukemic;
- (less frequently reported) diabetic, cirrhotic, asthmatic, and ulcerative colitis patients;
- the elderly;
- healthy people – some reports suggest that healthy people are at risk, and that antacids or cimetidine may predispose them to the infection. Some studies suggested that healthy, uncompromised people could develop the disease, particularly if the food eaten was heavily contaminated with *L. monocytogenes*.

6. Diagnosis

Identification of culture isolated from tissue, blood, cerebrospinal fluid, or another normally sterile site (e.g., placenta or fetus) is needed for diagnosis of *L. monocytogenes* infection. Stool cultures are not informative, since some healthy humans may be intestinal carriers of *L. monocytogenes*.

7. Food Analysis

Methods of analyzing foods for purposes of identifying *L. monocytogene*s are complex and time-consuming. The present FDA method, revised in January 2003, uses a single enrichment broth, buffered *Listeria* enrichment broth, and requires 24 to 48 hours of enrichment, followed by a variety of agars and, finally, biochemical confirmation. Total time to identification is from 5 to 7 days. Many other enrichment broths, such as UVM broth and Fraser broth, are also included in various protocols. Agars that have been extensively evaluated include Oxford agar, PALCAM, LPM plus esculin and ferric iron and MOX.

New molecular biology techniques have been used to develop various rapid-screening kits for *L. monocytogenes*. These kits generally rely on ELISA, PCR, and probe-based identification.

8. Examples of Outbreaks

L. monocytogenes has caused significant outbreaks worldwide over the past decades. Some examples are listed below.

Los Angeles, 1985. A large-scale listeriosis outbreak occurred in Los Angeles County, California, due to the consumption of contaminated Mexican-style soft cheese. Human listeriosis cases reported numbered 142. Among them, 93 cases occurred in pregnant women or their offspring, and the remaining cases occurred in non-pregnant adults. The outbreak led to 48 deaths, including 20 fetuses, 10 neonates, and 18 non-pregnant adults. An investigation of the cheese plant suggested that the cheese was commonly contaminated by unpasteurized milk. The outbreak strain was serotype 4b.

U.S., 1989, 2000. A serotype 1/2a strain was isolated from a single case of human listeriosis in 1989, which was caused by the consumption of processed meat. Eleven years later, the same strain isolated from sliced turkey produced by the same processing plant was implicated in a listeriosis outbreak. This provides a powerful illustration of *L. monocytogenes's* tenacity and prolonged survival in food-processing environments.

U.S., 1998 to 1999. A large scale multistate outbreak of listeriosis caused at least 50 cases in 11 states. Six adults died, and two pregnant women had spontaneous abortions. Contaminated hot dogs were linked to this outbreak. All *L. monocytogenes* isolates from these cases were serotype 4b.

U.S., 2002. A multistate outbreak of listeriosis in the Northeastern U.S. resulted in 46 cases, including 7 deaths and 3 stillbirths or miscarriages in eight states. The outbreak was linked to eating sliceable turkey deli meat. *L. monocytogenes* was isolated from 1 food product and 25 environmental samples from a poultry-processing plant. The isolate from the food product had a PFGE pattern different from the outbreak strain; however, two environmental isolates from floor drains had an identical PFGE pattern to that of outbreak patient isolates, suggesting that the plant might have been the source of the outbreak. The outbreak strain was serotype 4b.

Canada, 2008. A widespread outbreak of listeriosis occurred in Canada and was linked to deli meat produced by a Maple Leaf Foods plant in Toronto, Ontario. The outbreak caused 57 confirmed cases in seven provinces, and 22 people died. The outbreak strain was serotype 1/2a.

9. Resources

- Loci index for genome *Listeria*.
- GenBank Taxonomy database.
- CDC facts about listeriosis.
- The U.S. Department of Agriculture provides information about *Listeria monocytogenes*.

Bad Bug Book
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Mycobacterium bovis

1. Organism

Mycobacterium bovis, also referred to as *Mycobacterium tuberculosis* var. *bovis*, is a Gram-positive, aerobic, nonmotile, straight or slightly curved, rod-shaped bacterium that lacks an outer cell membrane. It does not have spores or capsules and is classified as an acid-fast bacterium, because in staining procedures its lipid-rich cell wall resists decolorization by acids.

Some other species of the genus *Mycobacterium* include *M. tuberculosis*, *M. leprae, M. africanum, M. avium*, and *M. microti*. Members of the *Mycobacterium* tuberculosis complex, which includes *M. tuberculosis* and *M. bovis*, are the causative agents of human and animal tuberculosis. *Mycobacterium bovis* is a causative agent of foodborne human tuberculosis (although it may also be transmitted via the airborne route, if it subsequently infects the lungs and results in active disease).

Mycobacterium species are considered hardy because of their unique cell walls, which enable them to survive long exposures to chemical disinfectants, including acids, alkalis, and detergents, and because they are able to resist lysis by antibiotics. *M. bovis* can survive in the environment for several months in cold, dark, moist conditions and for up to 332 days at a temperature range of 12°C to 24°C.

Some species of *Mycobacterium* are very difficult to grow (i.e., fastidious), unlike most bacterial pathogens, and could take up to 20 days to 2 years to culture. Optimum growth temperatures range from 25°C to more than 50°C. *Mycobacterium* species are referred to as rapid

For Consumers: A Snapshot

Tuberculosis most often spreads through coughing, but one type of bacterium can transmit the disease through contaminated food, usually **un**pasteurized ("raw") cow's milk or cheese or other food made from it. Read food labels to make sure milk and cheese say "pasteurized," which means that harmful bacteria have been killed.

Some people have no symptoms from foodborne tuberculosis; in others, it may take months or years for symptoms to appear. Symptoms include fever, night sweats, fatigue, loss of appetite, and weight loss. Foodborne tuberculosis sometimes affects different parts of the body and causes additional symptoms. For example, if it's in the intestines, it can cause diarrhea; if it's in the lungs, it can cause coughing (sometimes bloody). Tuberculosis can cause death, if it isn't treated with certain antibiotics for the right amount of time.

U.S. cattle- and food -inspection and prevention programs have kept the number of foodborne tuberculosis cases low in this country. The best way to protect yourself from foodborne tuberculosis is not to eat or drink raw cow's milk or foods made from it. Raw or undercooked meats from certain infected animals, including deer, also may cause tuberculosis if eaten. If you hunt or handle meats from animals like deer or elk, cook them thoroughly and wash your hands and disinfect kitchen surfaces that came into contact with the raw meat. Store the raw meat separately from other foods. U.S. prevention programs have greatly reduced the risk of tuberculosis from beef in this country, but it can also carry other harmful bacteria. Follow safe food-handling steps with any meat.

growers if they show visible growth colonies within 7 days, while those that require more than 7 days are referred to as slow growers. *M. bovis* is slow-growing in culture media.

Mycobacteria are widespread in nature, but the primary sources are water, soil, mastitic cows, and gastrointestinal tracts of animals. *Mycobacterium bovis* is pathogenic for cattle and some other animals, but also has been shown to be infectious to humans and, therefore, is a pathogen of concern to humans.

2. Disease

Mycobacterium bovis causes tuberculosis in cattle and is considered a zoonotic disease that also affects humans. Human tuberculosis caused by this organism is now rare in the United States, because of milk pasteurization and culling of infected cattle.

- **Mortality**: Death can result if the infection is left untreated. The Centers for Disease Control and Prevention (CDC) recently reported that an estimated three deaths (mean) are associated with *M. bovis* annually in the U.S.

- **Infective dose**: The infective dose of *Mycobacterium bovis* in cattle could be as low as 1 CFU (6-10 organisms), while the precise infective dose for humans is still unknown; it is suggested to be on the order of tens to millions of organisms.

- **Onset**: Symptoms generally appear months to years after initial infection. Some infected persons do not show any signs of the disease.

- **Illness/complications**: Ingestion of food contaminated with *M. bovis* can result in infection of the gastrointestinal tract or other parts of the body; for example, the lungs or the lymph nodes. (See Pathway section, below.) The disease may result in death, if untreated.

- **Symptoms**: Typical symptoms include fever, night sweats, fatigue, loss of appetite, and weight loss. Other symptoms depend on the part of the body affected; for example, chronic cough, blood stained-sputum, or chest pain, if the lungs are affected; or diarrhea, abdominal pain, and swelling, if the gastrointestinal tract is affected. Infections in humans also may be asymptomatic.

- **Duration**: Duration of illness depends on the immune status of the infected person. Symptoms could last for months or years, which necessitates a longer treatment period. Individuals with symptoms of lung involvement should avoid public settings until told by their health-care providers that they are no longer a risk to others.

- **Route of entry**: Mostly through ingestion (oral). Inhalation or direct contact with mucous membranes or broken skin, although not common, also are potential routes of exposure.

- **Pathway**: *M. bovis* can be taken up by alveolar macrophages in the lung, especially if transmission is by the aerosol route (pulmonary tuberculosis). From there it is carried to the lymph nodes, where the organism can migrate to other organs. *M. bovis* can multiply in these cells and in interstitial spaces, leading to formation of tubercle lesions. Gastrointestinal tuberculosis also causes the associated lymph nodes to form tubercles, although the organism may not spread to other organs.

3. Frequency

The CDC recently reported that an estimated 60 cases of human foodborne *M. bovis* tuberculosis (mean) are acquired in the U.S. each year.

4. Sources

Cattle and raw cow's milk are the typical sources of *M. bovis*; hence, food vehicles of particular concern include unpasteurized cow's milk and its products. The organism can be transmitted to humans through consumption of raw (unpasteurized) contaminated milk or other dairy products and raw or undercooked meat, such as venison, of infected animals. It can also be contracted through aerosol droplets; however this mode of transmission is less common, as is transmission via contact with the flesh of an infected animal (for example, via a wound or during slaughtering).

5. Target populations

All people are susceptible to *M. bovis* infection. Those who consume unpasteurized milk or products made with unpasteurized milk, especially young children, the elderly, and individuals with weak immune systems (for example, people with AIDS) are at higher risk of the disease than are others.

6. Diagnosis

Mycobacterium bovis is identified by isolating the bacteria from lymph nodes in the neck or abdomen, or from sputum produced by coughing. It is important to culture the organism in the laboratory and identify isolates according to cultural, biochemical, and molecular (DNA) techniques. Culturing and identification of *Mycobacterium bovis* are complicated and pose a risk of infection to laboratory personnel if safety procedures are not strictly followed.

7. Food analysis

Analysis of food for presence of *M. bovis* is challenging and complex. A variety of scientifically validated cultural, biochemical, and molecular techniques are utilized to identify *M. bovis* and distinguish it from other members of the *M. tuberculosis* complex. (See citation for Harris BN *et al*. in Resources section, below, for examples.)

8. Examples of Outbreaks

Michigan, 1994-2007. Two cases of tuberculosis due to *M. bovis* occurred. One case, in 2002, probably resulted due to consumption of unpasteurized milk by the patient or to the fact that the patient lived in a tuberculosis-endemic farm area. A second case occurred in 2004, due to exposure of the patient to infected deer. Investigations revealed that the outbreak strains related to the two human cases were genotypically the same as the deer/cattle outbreak strain.

New York City, New York, 2001-2004. This outbreak involved a multi-agency investigation in which there were 35 cases of human *M. bovis* illness in New York City. The investigation showed that fresh cheese from Mexico was implicated in the infection. One fatality was recorded (a 15-month-old boy).

9. Resources

Centers for Disease Control and Prevention. Human tuberculosis caused by *Mycobacterium bovis* – New York City, 2001-2004. MMWR Morb Mortal Wkly Rep. 2005;54:605–8.

Harris BN, Payeur J, Bravo D, Osorio R, Stuber T, Farrell D, Paulson D, Treviso S, Mikolon A, Rodriguez-Lainz A, Cernek-Hoskins S, Rast R, Ginsberg M, Kinde H. 2007. Recovery of *Mycobacterium bovis* from Soft Fresh Cheese Originating in Mexico. Appl. Environ. Microbiol. 73(3): 1025-1028.

Wilkins MJ, Meyerson J, Bartlett PC, Spieldenner SL, Berry DE, Mosher LB, *et al*. 2008. Human *Mycobacterium bovis* infection and bovine tuberculosis outbreak, Michigan, 1994–2007. Emerg Infect Dis Vol. 14, No. 4. April.

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Clostridium botulinum

1. Organism

Clostridium botulinum is an anaerobic, Gram-positive, spore-forming rod that produces a potent neurotoxin. The spores are heat-resistant and can survive in foods that are incorrectly or minimally processed.

Seven types of botulinum are recognized (A, B, C, D, E, F and G), based on the antigenic specificity of the toxin produced by each strain. Types A, B, E and F cause human botulism. (Types C and D cause botulism in animals. Types C and E also cause botulism in birds. No outbreaks of type G have been reported.) Most strains produce only one type of toxin, but strains producing dual toxin types have been reported.

The organism and its spores are widely distributed in nature. They are found in both cultivated and forest soils; bottom sediments of streams, lakes, and coastal waters; in the intestinal tracts of fish and mammals; and in the gills and viscera of crabs and other shellfish.

(Another species of *Clostridium*, i.e., *perfringens*, causes foodborne illness, but does not cause botulism. It is addressed in another chapter.)

2. Disease

Overview: Botulism is a serious, sometimes fatal, disease caused by a potent neurotoxin formed during growth of *C. botulinum*. The infection results in flaccid paralysis of muscles, including those of the respiratory tract. Three major types of botulism are known, two of which will be discussed in this chapter: foodborne

For Consumers: A Snapshot

Not many people get botulism – the illness this bacterium causes – but when they do, it's often deadly *if it's not treated*, although some cases can be mild. A toxin produced by the bacterium causes the illness. The bacterium grows well in places with low oxygen, such as cans of food that became contaminated before being sealed. Often, there's no visible sign that a food is contaminated, but sometimes a can is swollen. Most often, illnesses are due to home-canned foods that weren't processed or cooked properly. Occasionally, canned foods sold in stores have caused botulism. Tiny amounts of the toxin can cause paralysis, including paralysis of the breathing muscles. With anti-toxin and other treatment, and the help of a "breathing machine," the paralysis usually goes away within weeks or, in severe cases, months. Early symptoms start from 4 hours to 8 days after eating (although it's usually 18 to 36 hours) and include double or blurred vision, drooping eyelids, slurred speech, swallowing problems, dry mouth, muscle weakness, constipation, and swollen abdomen. You can help protect yourself from botulism by following canning instructions and good hygiene if you make home-canned foods and by boiling canned foods for 10 minutes before eating them, whether they're home-made or store-bought.

A special type of botulism, infant botulism, occurs when the bacterium "sets up housekeeping" in babies' intestines and makes the toxin there, in the gut. Constipation is often the first sign. Other symptoms are dull face, weak sucking, weak cry, less movement, trouble swallowing, more drooling than usual, muscle weakness, and breathing problems. ***Children under 1 year old should never be fed honey***, which has been linked to infant botulism (but not to adult botulism). It's important to give early treatment with an anti-toxin made especially for infant botulism.

botulism and infant botulism, which also is foodborne. The third type, wound botulism, is not foodborne and will not be covered extensively in this chapter.

Botulinum toxin causes flaccid paralysis by blocking motor nerve terminals at the neuromuscular junction. The flaccid paralysis progresses symmetrically downward, usually starting with the eyes and face, to the throat, chest, and extremities. When the diaphragm and chest muscles become fully involved, respiration is inhibited and, without intervention, death from asphyxia results.

> ***Foodborne botulism*** is a severe type of food poisoning caused by ingestion of foods containing the toxin produced by *C. botulinum*. This type of botulism most often develops after consumption of improperly processed and inadequately cooked home-preserved foods. Home-canned or, occasionally, commercially produced foods have been involved in botulism outbreaks in the United States. Although the incidence of the disease is low, the disease is of considerable concern because of its high mortality rate if not treated immediately and properly.
>
> ***Infant botulism*** is a serious illness caused by ingestion of *C. botulinum* spores that colonize and produce toxin in the intestinal tracts of infants (i.e., intestinal toxemia botulism).
>
> ***Wound botulism*** is the rarest form of botulism and is discussed only briefly here, because it does not involve food. It results when *C. botulinum* colonizes in a wound and produces toxins, which reach other parts of the body via the bloodstream. Whereas foodborne botulism is limited to the amount of toxin ingested, *C. botulinum* in wounds produce toxin *in situ* (gas gangrene) until the pathogen is gone.

A fourth, "undetermined" category consists of adult cases in which a food or wound source cannot be identified. It has been suggested that some cases of botulism assigned to this category might result from intestinal colonization in adults, with *in vivo* production of toxin.

The medical literature suggests the existence of an adult form of botulism similar to infant botulism. In these cases, patients have had surgical alterations of the gastrointestinal tract and/or antibiotic therapy. It is proposed that these procedures may have altered the normal bacterial population of the gut and allowed *C. botulinum* to colonize the intestinal tract.

Recommended treatment for foodborne botulism includes early administration of botulinum antitoxin, available from the Centers for Disease Control and Prevention (CDC), and intensive supportive care, including mechanical breathing assistance. An antitoxin for infant botulism (Botulism Immune Globulin Intravenous, abbreviated BIG-IV) also is available and should be administered as early in the illness as possible. Antimicrobial therapy is not recommended, due to concerns about increased toxin release as a result of cell lysis.

- **Mortality:** The mortality rate is high if treatment is not immediately administered. The disease is generally fatal in 5% to 10% of cases.
- **Infective dose**: An extremely small amount – a few nanograms – of the toxin can cause illness.

- **Onset**:

  Adult: Usually 18 to 36 hours after ingesting food containing the toxin, although times have varied from 4 hours to 8 days.

  Infant: Generally follows a period of normal development.

- **Illness / complications:** See above.

- **Symptoms:**

  Adult: Initial symptoms may include double vision, blurred vision, drooping eyelids, slurred speech, difficulty swallowing, dry mouth, and muscle weakness. If the disease is not treated, symptoms may progress to paralysis of the arms, legs, trunk, and respiratory muscles.

  Early signs of intoxication consist of marked lassitude, weakness and vertigo, usually followed by double vision and progressive difficulty in speaking and swallowing. Difficulty in breathing, weakness of other muscles, abdominal distention, and constipation may also be common symptoms

  Infant: Constipation after a period of normal development is often the first sign of infant botulism. This is followed by flat facial expression; poor feeding (weak sucking); weak cry; decreased movement; trouble swallowing, with excessive drooling; muscle weakness; and breathing problems.

- **Duration**: Patients with severe cases that involve paralysis of the respiratory muscles may need mechanical ventilation and intensive care for weeks or months.

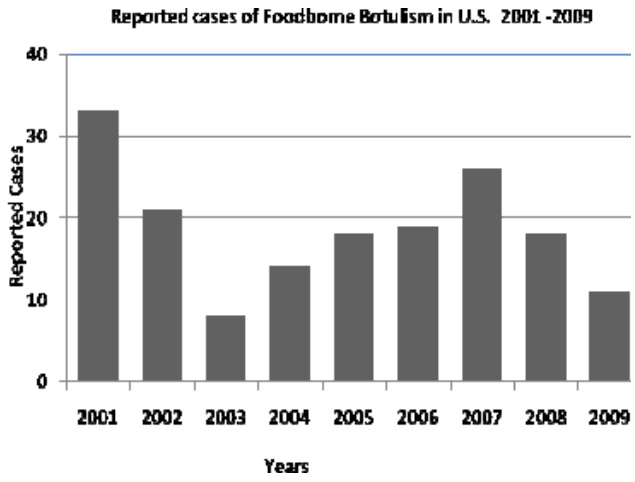
- **Route of entry**: Oral, for foodborne infection. (Infection of wounds also occurs).

- **Pathway:** Clinical presentation develops after a person ingests the pre-formed toxin, or if the organisms grow in the intestines or in wounds, followed by toxin release. The ingested botulinum toxin (an endopeptidase enzyme) blocks peripheral cholinergic neurotransmission at the neuromuscular junction and cholinergic autonomic nervous system. The toxin acts by binding presynaptically to high-affinity recognition sites on the cholinergic nerve terminals and decreasing the release of acetylcholine, causing a neuromuscular blocking effect. (This mechanism laid the foundation for development of the toxin as a therapeutic tool; e.g., when temporary inactivation of specific muscles is needed for therapeutic or cosmetic purposes.)

  C. botulinum produces the toxin as a complex of proteins, among which is the neurotoxic moiety. The toxin is synthesized as a relatively inactive single-chain polypeptide with a molecular weight of ~150 kDa. It becomes an active toxin by selective proteolytic cleavage to yield the heavy and light chains that are linked by a single disulphide bond and non-covalent interactions. The toxin's light chain is a Zn^{++}-containing endopeptidase that blocks acetylcholine-containing vesicles from fusing with the terminal membrane of the motor neuron, resulting in flaccid muscle paralysis.

3. Frequency

As noted, the incidence of the disease is low, but the mortality rate is high, if the disease is not treated immediately and properly. Some cases of botulism may go undiagnosed because symptoms are transient or mild or are misdiagnosed as Guillain-Barré syndrome.



Source: National Botulism Surveillance, Centers for Disease Control and Prevention

4. Sources

General info: The types of foods involved in botulism vary according to food preservation and cooking practices. Any food conducive to outgrowth and toxin production can be associated with botulism. This can occur when food processing allows spore survival and the food is not subsequently heated before consumption, to eliminate any live cells.

Almost any type of food that is not very acidic (pH above 4.6) can support growth and toxin production by *C. botulinum*. Salt concentration from 4% to 5% is needed for inhibition of its spores (especially regarding type E), with a 5% concentration completely inhibiting their growth. Salt concentrations slightly lower than those providing inhibition tend to extend spore outgrowth time at low temperatures.

A variety of foods, such as canned corn, peppers, green beans, soups, beets, asparagus, mushrooms, ripe olives, spinach, tuna fish, chicken and chicken livers, liver pate, luncheon meats, ham, sausage, stuffed eggplant, lobster, and smoked and salted fish have been associated with botulinum toxin.

Infant botulism*:** Of the various potential environmental sources, such as soil, cistern water, dust, and foods, honey is the one dietary reservoir of *C. botulinum* spores linked to infant botulism by both laboratory and epidemiologic studies. ***Honey should not be fed to infants under 12 months of age.

5. Target Populations

All people are believed to be susceptible to botulism.

6. Diagnosis

Although botulism can be diagnosed by clinical symptoms alone, differentiation from other diseases may be difficult. The most direct and effective way to confirm the clinical diagnosis of botulism in the laboratory is to demonstrate the presence of toxin in the serum or feces of the patient or in the food the patient consumed. Currently, the most sensitive and widely used method for detecting toxin is the mouse neutralization test. This test takes 48 hours. Culturing of specimens takes 5 to 7 days.

7. Food Analysis

Since botulism is foodborne and results from ingestion of preformed *C. botulinum* toxin, determination of the source of an outbreak is based on detection and identification of toxin 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 detection and isolation of the causative organism.

8. Examples of Outbreaks

See CDC information on surveillance and investigation.

9. Other Resources

Loci index for genome *Clostridium botulinum* is available from GenBank.

CDC's Emergency Preparedness and Response for Botulism.

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Foodborne Pathogenic Microorganisms and Natural Toxins

Enterococcus

1. Organism

Enterococci are Gram-positive, catalase-negative, facultative anaerobes that normally are spherical and ovoid, are less than 2 μm in diameter, and occur in chains or pairs or singly. They are opportunistic pathogens and, in general, are able to survive harsh conditions. Virulence factors in enterococci include cytolysin/hemolysin, gelatinase, serine protease, adhesins, and enterococcal surface protein.

Optimal growth temperature for enterococci is 35°C. They may grow at temperatures up to 45°C, and reports of minimum growth temperature have varied from 10°C to 20°C. They can grow in 6.5% NaCl broth and in broth with a relatively high pH of up to 9.6. Enterococci hydrolyze esculin in a medium containing 40% bile salts. These characteristics may be used for isolation and identification of the bacterium. At the same time, they present a challenge for control of pathogenic isolates.

For Consumers: A Snapshot

Anyone can become infected with the *Enterococcus* bacterium, but the people most likely to suffer serious problems are those who already have other serious illnesses. In otherwise healthy people, it may cause diarrhea, cramps, nausea, vomiting, fever, and chills, starting 2 to 36 hours after they eat contaminated food. *Enterococcus* can be passed to people in different ways, and not much is yet known about how often it's transmitted by food. But it is known that meat and milk that aren't processed or cooked properly or that are handled in unsanitary ways are among the foods that can transmit it. A major concern about *Enterococcus* is that it has become resistant to some antibiotics that were used to treat it – that is, those antibiotics no longer kill it. You can help protect yourself from getting foodborne illness from this and other bacteria or viruses by following basic food-safety tips; for example, by ***not*** using **un**pasteurized ("raw") milk or certain cheeses and other food made from it, by thoroughly cooking meat or food that contains meat, and by washing your hands, kitchen equipment, and other surfaces before and after you handle food.

Most bacteria in the genus *Enterococcus* used to be classified as Group D *Streptococcus*, mainly of fecal origin. However, molecular phylogenetic analysis has revealed that *Enterococcus* spp. are more closely related to *Vagococcus*, *Tetragenococcus*, and *Carnobacterium* than to *Streptococcus*; thus, in 1984, the new genus *Enterococcus* was established.

Both conventional microbiologic and modern molecular methods have been used for the classification and identification of *Enterococcus* species. At least 27 species have been proposed for inclusion in the genus. *Enterococcus faecalis* is the predominant enterococcal species recovered from animal and human feces and clinical specimens, followed by *Enterococcus faecium*.

Enterococci are also divided into five physiological groups, by most laboratories, based on acid formation in mannitol and sorbose broths and hydrolysis of arginine. The grouping information is very useful for medical personnel. Identification of enterococci to species level by

conventional methods can take up to 10 days. However, identification of enterococci to group level can be done in 2 days, in most cases.

The toxin cytolysin is produced by some *E. faecalis* strains. It displays both hemolytic and bactericidal activities.

2. Disease

Enterococcus infection is notable largely as a hospital- or community-acquired illness; not much information is available regarding the foodborne illness. The organism is not on the list of Centers for Disease Control and Prevention (CDC) notifiable diseases or on the outbreak summary report. In general, *Enterococcus* poses a serious threat mainly to people who are debilitated by other, grave underlying conditions.

The emergence and global spread of vancomycin-resistant and multi-drug resistant enterococci pose a major threat to human heath and have serious implications for health care. Research suggests that clinically relevant gentamicin-, glycopeptide-, and streptogramin-resistant enterococci can be transmitted to humans via consumption of food animals.

Overall, however, the dearth of data on *Enterococcus* transmitted by food requires that the information in this Disease section of the Bad Bug Book be approached with the understanding that it is unknown how much of it applies to foodborne *Enterococcus*.

- **Mortality:** Enterococcal infections are not a leading cause of death in the United States. However, the mortality rate for bacteremia associated with *Enterococcus faecium* may be as high as 50%. The mortality rate for *endocarditis* – a potential consequence of Enterococcus infection – is 15% to 20%.

- **Infective dose**: An infective dose of at least 107 cells has been reported for *Streptococcus D*, the organism under which *Enterococcus* originally was classified.

- **Onset**: Two to 36 hours after contaminated food is ingested.

- **Disease / complications:** Enterococci, in general, can cause many different infections, such as endocarditis, bacteremia, urinary tract infections, intra-abdominal and pelvic infections, and nosocomial infections, and neonatal infections. Ampicillin, penicillin or vancomycin, ureidopenicillin, streptomycin, and gentamicin are used singly or in combination for treatment of various enterococcal infections.

- **Symptoms**: Symptoms may include diarrhea, abdominal cramps, nausea, vomiting, fever, chills, dizziness. The infection may produce a clinical syndrome similar to staphylococcal intoxication (see chapter on *Staphylococcus aureus*).

- **Route of entry**: Oral.

3. Frequency

Reports regarding frequency of enterococcal infections associated with foods in the U.S. alone or elsewhere are limited. However, it is reported that, in human clinical specimens, about 80% to 90% of enterococcal infections were caused by *E. faecalis* and 5% to 20% by *E. faecium*. Several enterococcal species less frequently isolated from humans include, for example,

E. avium, E. casseliflavus, E. cecorum, E. dispar, E. durans, E. gallinarum, E. gilvus, E. hirae, E. mundtii, E. pallens, and *E. raffinosus*, which have been detected in clinical samples.

4. Sources

Because enterococci can grow and survive in harsh environments, they are almost ubiquitous in nature. They can be found in animals, birds, insects, plants, soil, and water. They are commensal bacteria in human and animal gastrointestinal tracts, and less common in other parts, such as the genitourinary tracts, oral cavities, dental plaque, and skin. Some enterococci have been used as starters in cheese-making and in other fermented milk products.

Examples of food sources have included sausage, evaporated milk, cheese, meat croquettes, meat pie, pudding, raw milk, and pasteurized milk. Entrance into the food chain is often due to under-processing of food or unsanitary food preparation.

5. Diagnosis

Culturing of stool samples, blood, and suspect food is recommended. *Enterococcus* spp. generally grow well on blood-based media. Usually, 5% sheep, horse, or other animal blood are added to the media. Molecular methods, such as polymerase chain reaction (PCR), also are available.

6. Target Populations

All people are susceptible. However, people with serious underlying conditions, children, and people 65 years and older, in nursing homes, are more vulnerable.

7. Food Analysis

Suspect food is examined microbiologically by nonselective and selective medium techniques, which can take up to 7 days. Group specificities are determined by Lancefield group-specific antisera. Both biochemical and DNA techniques are used for identification.

8. Examples of Outbreaks

For more information about recent outbreaks, see Morbidity and Mortality Weekly Report (MMWR) from CDC.

9. Resources

Taxonomy and DNA sequence information for *Enterococcus*

Parasitic Protozoa and Worms

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Toxoplasma gondii

1. Organism

Toxoplasma gondii are obligate intracellular parasites that belong to the family Sarcocystidae. *T. gondii* is ubiquitous and is found around the world. In some locations, up to 95% of the local human population is or has been infected with *T. gondii*. These locales are often in hot, humid climates and lower altitudes.

T. gondii has a complex life cycle that includes a separate asexual and sexual cycle and involves a definitive and an intermediate host. The asexual cycle occurs in a wide range of intermediate hosts that include warm-blooded vertebrates, such as birds, carnivores, rodents, pigs, primates, and humans. The sexual cycle occurs exclusively in the definitive host, the wild and domestic feline. Domesticated cats are the principal definitive hosts and shed infectious oocysts in their feces. Oocysts are resistant to the effects of many environmental factors and can persist for several years, under certain conditions.

Other important stages of the life cycle of *T. gondii* include bradyzoites and tachyzoites. Oocysts must undergo sporulation, forming bradyzoites that are released by proteolytic enzymes in the host. This form then invades intestinal epithelial cells and is transformed into tachyzoites, which are capable of dissemination through blood or lymph. It is this form that can also transit the placenta. The disease this pathogen produces is toxoplasmosis.

T. gondii was first described by Charles Nicolle and Louis Manceaux in 1908, in Tunis, Tunisia, and later that year also was described, in Brazil, by Alfonso Splendore.

For Consumers: A Snapshot

According to the CDC, this parasite is the second leading cause of death from foodborne illness in the U.S. The illness it causes, toxoplasmosis, can be serious or deadly, particularly for babies infected in the womb and people with weak immune systems (such as people with AIDS or people on medications that lower immunity, like some drugs for arthritis or cancer treatment.) Infected people who are otherwise healthy often have mild symptoms or none. People can get infected (1) by eating raw or undercooked, contaminated meat – not only meats like pork and beef, but also seafoods, like clams and oysters; (2) when contaminated cat feces (even particles too small to see) get on their hands, objects, or food, or in water, and end up in their mouths – for example, if they clean a cat's litter box or work in the garden and don't wash their hands well; (3) by passing the parasite from a mother to her unborn baby – the mother may not get sick, but the baby often dies, or, if born, has serious health problems; or (4) from contaminated blood transfusions or transplanted organs. No matter how it's passed on, this parasite could make anyone very sick. It can spread to other parts of the body, such as the brain, eyes, or heart and other muscles. Seizures, vision loss, or heart and lung problems are just a few potential outcomes of infection. Most infections are treated with sulfa drugs. You can prevent infection by cooking all meats well; washing fruits and vegetables before eating them and handling meats and other foods safely; drinking, cooking, and cleaning only with sanitary water; washing your hands well after handling cats, litter boxes, and soil; and avoiding **un**pasteurized ("raw") milk, especially goat's milk. These precautions are especially important if you're pregnant or have a weak immune system.

T. gondii is considered a formidable foodborne pathogen in the United States. In a 2011 report (Scallan *et al*., 2011) by the Centers for Disease Control and Prevention (CDC), *T. gondii* is listed as a prominent etiological agent that causes an estimated 86,686 foodborne- related illnesses in the U.S. annually. Of note: this pathogen causes approximately 4,428 hospitalizations per year and is responsible for nearly 327 deaths per year, ranked second, behind *Salmonella*. *T. gondii* infections can also lead to acute ocular disease; nearly 4,800 cases per year.

More than 60 million men, women, and children in the U.S. are estimated to carry *T. gondii*; most are asymptomatic. However, *T. gondii* infections in pregnant women pose a serious health concern to the fetus. When a woman becomes infected with *T. gondii*, either at conception or during pregnancy, the parasite can cross the placenta and infect the fetus. These neonates can either be asymptomatic, or the infection can lead to a range of disabilities; e.g., hydrocephalus, developmental delay, or death. Pregnant women and immunosuppressed people should avoid consuming raw or undercooked meat, unpasteurized goat's milk, and untreated water. Toxoplasmosis is not transmitted via person-to-person. The notable exception is the transplacental transfer of the parasite of pregnant women to their fetuses, via congenital transmission; blood transfusion; or organ transplantation.

Prevention of *T. gondii* infection depends on a number of factors. Since cats are the primary source of transmission, avoidance or proper precautions when handling cat feces (litter boxes) is recommended. Most important, thorough hand washing before and after handling foods (preparation), before eating, and after working with soil and cleaning litter boxes can help in preventing toxoplasmosis.

This parasite can withstand environmental stresses. Oocysts can remain infective for approximately 150 to 400 days in water at temperatures ranging from 4 to 37ºC. Similar observations have been made of oocysts in soil. Desiccation (drying) can also be a factor; oocyst infectivity persists longer in a moist environment than in a dry region.

Tissue cysts containing *T. gondii* are likewise affected by temperature. Exposure to temperatures at or below -13^{o}C, for at least 24 h, will usually kill cysts. Meats should be cooked at selected temperatures to ensure that any *T. gondii* cysts are killed. The USDA recommends that meats be cooked at 68.2ºC (155ºF) or higher, with a 3-minute rest. Ground meat and wild game meats should be heated at 71.1ºC (160ºF) or higher, whereas poultry should be cooked at 73.9ºC (165ºF) or higher. It appears that microwave heating of any of the above meats is not reliable for killing all of the *T. gondii* cysts. For further information about preventing *T. gondii* infections from foods, see the Jones and Dubey reference below.

2. Disease

- **Mortality**: *T. gondii* is a ubiquitous parasite that can have significant morbidity and mortality in congenitally infected fetuses and in immunocompromised individuals. The disease is usually self-limiting, but can be fatal to a fetus via a mother who ingested the parasite, usually after becoming pregnant, leading to miscarriage or stillbirth. If left untreated, toxoplasmosis can impose a high mortality rate.

- **Infective dose:** Not known.

- **Onset**: Five to 23 days after ingestion from contaminated food, water, and fingers.

- **Illness / complications**: Women who become infected during pregnancy typically are asymptomatic, although the parasite (tachyzoites) can cross the placenta. In infected pregnant women, the fate of the fetus falls into three possibilities: miscarriage or stillbirth; head deformities; or brain or eye damage. Some of the clinical manifestations of *T. gondii* infections in the unborn include hydrocephalus or microcephaly, intracranial calcification, and chorioretinitis.

  A newborn may become infected at birth with *T. gondii*, but not show any apparent symptoms of infection. However, latent infections in these individuals may cause loss of vision, when they are adults (ocular toxoplasmosis); seizures; or mental disabilities. In addition, immunocompromised individuals may develop pneumonitis, retinochoroiditis, brain lesions, and central nervous system diseases.

  Sulfa compounds, specifically sulphadiazine and pyrimethamine, are the chemotherapeutics of choice for treating toxoplasmosis. These drugs are usually well tolerated by infected individuals and are most effective when administered during the acute stage of infection (active multiplication of the parasite). Although treatment with sulfonamides will limit tissue cyst growth, they appear to have little effect on subclinical infections and usually will not eradicate infection.

- **Symptoms**

  In acute toxoplasmosis, sore lymph nodes and muscle pains develop in 10-20 % of patients and can last for several weeks, after which symptoms no longer are exhibited. Symptoms of ocular toxoplasmosis are blurred or reduced vision, tearing of, or redness in, the eye; pain; and sensitivity to light. In healthy individuals, toxoplasmosis usually is asymptomatic. In some cases, flu-like symptoms may appear, such as swollen lymph glands, fever, headache, and muscle aches. Death is rare in acute cases. In immunocompromised individuals, such as patients with organ transplants, AIDS, or cancer, or who are taking immunosuppressive medications, toxoplasmic encephalitis is a common clinical manifestation that can be fatal. Additional symptoms include confusion, nausea, poor coordination, and seizures. Several published reports (see Torrey *et al.*, below) suggest a link between prenatal infection with toxoplasmosis and the potential for schizophrenia later in life. Individuals who progress to chronic infections may develop cysts in various muscle tissues, including brain, heart, and skeletal tissues.

- **Duration**: Infected, but otherwise healthy, individuals often display no symptoms; the host's immune system usually keeps the parasite in check and prevents it from causing illness. If illness does occur, mild flu-like symptoms (acute phase) usually last for several weeks, but can fade in a few days to months, then disappear. However, the *T. gondii* parasite can remain in the host as tissue cysts (bradyzoites), but in this latent state are inactive. Reactivation of bradyzoites can occur under certain circumstances, such as when an infected person becomes immunosuppressed by other diseases or medication; under these conditions, toxoplasmosis (chronic) can develop.

- **Route of entry**: The primary routes of entry are (1) consumption of undercooked or raw, parasite-encysted (with bradyzoites) meats (e.g. lamb; goat; pork; poultry; game meats, such as venison; horse), (2) ingestion of contaminated water or soil with cat feces or through the fecal-oral route, via fomites (e.g., knives, utensils, cutting boards), fingers, or other foods that had contact with raw, contaminated meat, (3) congenital transmission, in

which a pregnant mother passes the parasite to the fetus via transplacental transmission, and (4) possibly through raw oysters, clams, and mussels.

- **Pathway**: *Toxoplasma gondii* has a very complex life cycle, in which cats (or Felids) are the primary or "definitive" host; the animal in which parasitic sexual reproduction occurs to produce infective parasitic life forms. Cats become infected with *T. gondii* by eating sporulated (mature) oocysts from contaminated environmental sources or *T. gondii*-encysted tissue sources (such as rodents). Large numbers of immature oocysts shed by infected cats require several days in the environment to sporulate and become infective. Oocysts are very resistant to most environmental conditions and can survive for more than a year outside of the definitive host. Intermediate hosts, *i.e.* humans, birds, pigs, sheep, goats, and cattle, become infected after eating food or drinking from contaminated water sources.

  In the cat, the mature oocyst is degraded to release infectious organisms into the intestine. Some will invade and replicate within intestinal epithelial cells; others will penetrate through the intestinal wall and replicate throughout the body. These fast-replicated forms are called tachyzoites. Tachyzoites that develop within the intestinal epithelium will differentiate into sexual forms, fertilize, and develop into immature oocyts that are then shed into the environment. Infected cats will begin shedding within 3 to 10 days, and this will last for 10 to 14 days. Tachyzoites that eventually localize in tissue other than the intestinal epithelium will transform (because of an immune response by the cat) into bradyzoites contained within tissue cysts.

  Infection of the intestinal epithelium does not occur in the intermediate host. As in the cat, parasites will penetrate the intestinal epithelium and migrate throughout the host as tachyzoites. Tissue infection and the host immune response will cause the formation of bradyzoite-filled tissue cysts. These will remain viable throughout the life of the host, and these encysted bradyzoites are infectious to cats and other intermediate hosts. In acute infections in humans, intestinal epithelial cells are the primary site of invasion, with potential subsequent spread to other sites, such as the brain, heart, and skeletal muscle.

3. Frequency

Worldwide, the number of people infected is estimated to be more than 30%. However, variations occur from country to country. In France and Germany, most of the population may be infected by this parasite, whereas in some countries, such as South Korea, it is quite rare for anyone to be infected. In the U.S., more than 60 million people may be infected; approximately 22.5% of the population. *T. gondii* infections are found in 500 to 5,000 newborns in the U.S.; not a surprising number, given that approximately 89% of women of childbearing age may have been exposed to this parasite and can pass it to their newborn children, particularly if the woman has a primary infection during pregnancy.

4. Sources

The primary source of the *T. gondii* parasite is feces from domestic and wild cats (Felidae). Cats are the only animal that sheds oocysts in its feces and contaminates the environment with them. Transmission occurs from environmental contact, through intermediate hosts, such as birds, rodents, pigs, sheep, and cattle; *e.g.*, feeding on oocyst-contaminated feed, water, or soil.

The primary mode of foodborne transmission is ingestion of undercooked or raw meats (e.g., pork, lamb, or wild game) containing tissue cysts from a chronically infected host or by the consumption of food (fruits and vegetables) contaminated with cat feces or drinking of water containing sporulated oocysts. In addition, placing hands to mouth after handling cats, their litter box or feces, or anything that may have come in contact with their feces is a source of transmission. Any material that comes in contact with parasite-laced feces is at risk of being contaminated.

5. Diagnosis

Diagnosis of *T. gondii* typically is performed by serological testing that targets the immunoglobulin IgG and IgM, available in different formats: enzyme-linked immunosorbent assays (ELISA), immunoblots, dye test, and indirect fluorescent antibody test. These tests typically detect the host's antibodies generated within several weeks of infection and, in some cases, estimate the length of infection, as this is an important factor regarding pregnant women. In the latter, the target immunoglobulin is IgM. In some cases, direct observation of the parasite in stained tissue samples, cerebrospinal fluid (CSF), or other biopsy material can be performed, although it is very time-consuming and, in some cases, difficult to extract a specimen. Molecular techniques are used for detecting *T. gondii* DNA in the amniotic fluid in cases of congenital transmission (mother-to-child transmission). Ocular toxoplasmosis diagnosis usually is based on symptoms, appearance of lesions in the eye, serologic testing, and observation of the infection's course.

6. Target populations

Infection is usually asymptomatic, with no obvious symptoms in immunocompetent and otherwise healthy individuals. When symptoms do occur, such as fatigue, flu-like symptoms, muscle aches and pains, and swollen glands, they are usually mild and short-lived. Those most affected are individuals with an impaired immune system and pregnant women. In such cases, illness may be life-threatening, particularly to a developing fetus.

7. Food Analysis

Analysis of foods usually is achieved by serology, although tissue cysts may be observed in stained biopsy specimens from infected meats.

8. Examples of Outbreaks

Parasites like *T. gondii* usually will enter the food chain via three scenarios. These include direct contamination of food ingredients or farm-fresh produce; through contaminated water sources used in irrigation, washing, or processing of foods; and through direct human transfer by food handlers or processors or in the home.

Epidemiologic evidence suggests that most outbreaks of illness in humans occur through consumption of uncooked or undercooked meat containing viable tissue cysts. Documented outbreaks have been described in which the ingestion of infected meat, such as uncooked pork, was the major source of infection. However, large-scale outbreaks linked to municipal water sources and consumption of unfiltered water have altered such thinking. This includes outbreaks in Canada, in 1994, attributed to a contaminated municipal water supply, and in several regions of Brazil.

9. Other Resources

Genome link

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Bad Bug Book

Foodborne Pathogenic Microorganisms and Natural Toxins

Giardia lamblia

1. Organism

Giardia lamblia (also referred to as *Giardia intestinalis* or *Giardia duodenalis*) is a single-celled, enteric protozoan parasite that moves with the aid of five flagella, which also assist with attachment to intestinal epithelium.

Giardia is infective in the cyst stage, when it is also extremely resistant to environmental stressors, including cold temperatures and chemicals. As noted in the Sources section, below, chlorine concentrations typically used for post-harvest rinsing do not kill the cysts, which are hard to wash off of types of produce that don't have smooth surfaces. The Sources section also notes that infected food handlers often are implicated in outbreaks.

Reservoirs for *Giardia* include the intestine of infected humans or other animals (e.g., cats, dogs, cattle, deer, and beavers). Organisms that appear identical to those that cause human illness have been isolated from domestic animals (dogs and cats) and wild animals (beavers, muskrats, bears). A related, but morphologically distinct, organism infects rodents, although rodents may be infected with human isolates in the laboratory.

2. Disease

Giardiasis is a very frequent cause of non-bacterial diarrhea in North America and is one of the most

For Consumers: A Snapshot

Giardia (pronounced "jee-AR-dee-ah") is a parasite that can make people sick when they eat or drink contaminated food or water. In some cases, there are no symptoms, but, often, people who have giardiasis (the illness caused by *Giardia*) have diarrhea that smells especially bad, gas, nausea, cramps, vomiting, and weight loss. People who have giardiasis and who prepare food may contaminate the food if they don't wash their hands well after going to the bathroom, for example. The people who eat the food may then get giardiasis. A person with poor hygiene can pass *Giardia* to another person through direct contact; for example, if *Giardia* gets on the hands, and then into the mouth, of the other person. Children in day-care centers often get giardiasis in this way. On the farm, contaminated water can contaminate crops. Even fresh streams in the wilderness may be contaminated with *Giardia*, from animals that pass it in their bowel movements. Most common chlorine preparations don't kill *Giardia*, so it can live in some swimming pools or other water used for recreation. Giardiasis may go away by itself within 2 to 6 weeks in most people who are otherwise healthy, although it may last much longer in others. Especially for those people, getting medications from a health professional, to stop the illness, is important. Dehydration is a concern, especially for young children. Anyone can get giardiasis, and those at higher risk include hikers, hunters, and others who might drink water from the outdoors; and children in day-care centers. You can help protect yourself and others from *Giardia* by washing your hands well after going to the bathroom or cleaning someone else who has gone to the bathroom, and after handling pets, diapers, soil, and outdoor water, from puddles to rivers. You can kill *Giardia* at its infectious life stage by boiling water for 5 minutes. Outdoor-recreation stores sell filters that remove *Giardia* from water.

commonly isolated enteric protozoans in clinical specimens. Routes of transmission include contaminated water, food, and person-to-person contact with someone who is ill with giardiasis, especially when adequate fecal-oral hygiene is lacking.

- **Mortality**: Giardiasis generally is not associated with mortality in otherwise healthy people.

- **Infective dose:** Ingestion of one or more cysts may cause disease.

- **Onset**: Usually 1 to 2 weeks after ingestion of a cyst(s).

- **Illness / complications:** Giardiasis is self-limiting in most people. However, some (less than 4%) remain symptomatic for more than 2 weeks, possibly leading to a malabsorption syndrome and severe weight loss. Severe dehydration due to loss of fluids is a major concern, especially in young children. Malabsorption of vitamins, protein, and iron all are possible with chronic infections, and it has been suggested that, in children, this can result in stunted growth and development. Chronicity of infection is correlated with an absence of secretory IgA in the intestinal lumen.

  About 40% of those who are diagnosed with giardiasis develop disaccharide intolerance during infection and up to six months after resolution of infection. Lactose (i.e., milk sugar) intolerance is most frequently observed due to intestinal epithelial cell brush border damage by the *Giardia* trophozoites.

  Several strains of *G. lamblia* have been isolated and described through analysis of their proteins and DNA; type of strain, however, is not consistently associated with disease severity. Different people infected with the same strain have various degrees of symptoms, and the symptoms of an individual may vary during the course of the disease.

  Flagyl (metronidazole) is normally quite effective in terminating infections and is the first-line choice. However, treatment lasts for up to 7 days, and substantial side effects are not uncommon. Tinidazole (brand names: Tindamax and Fasigyn) is another effective drug against giardiasis, as it inhibits DNA synthesis. In some patients, it is better tolerated than is flagyl, due to fewer side effects and because treatment is given in a single dose. Chronic cases of giardiasis are frequently refractory to drug treatment. In some immune-deficient individuals, giardiasis may contribute to a shortening of the life span. Prophylactic treatment usually is not considered.

- **Symptoms**: Infections sometimes are asymptomatic. When symptoms are present, they generally consist of especially malodorous diarrhea, malaise, abdominal cramps, flatulence, and weight loss.

- **Duration**: Generally 2 to 6 weeks, unless the illness becomes chronic, in which case it may last for months or years and may become difficult to treat.

- **Route of entry**: Oral

- **Pathway**: The mechanism by which *Giardia* causes disease is largely unknown. Investigators have been unable to confirm reports that the organism produces a toxin. Infrequently, it has been found in the duodenal cells of its hosts, but this probably is not responsible for the symptoms of the disease. The organism has been found inside host

duodenum cells, but this is an infrequent occurrence that is, more than likely, not responsible for disease symptoms. Mechanical obstruction of the absorptive surface of the intestine has been proposed as a possible pathogenic mechanism.

3. Frequency

In 2002, giardiasis became a nationally notifiable disease. The overall incidence of infection in the United States is estimated to be 2% of the population. Asymptomatic infections are largely undocumented, yet are known to occur. Giardiasis surveillance in the U.S. from 1998-2005 showed a range of 19,708 to 24,226 reported cases annually, which were distributed throughout nearly all U.S. jurisdictions. According to a more recent estimate by the Centers for Disease Control and Prevention (CDC), 76,840 cases of giardiasis (most of them from contaminated water, but some from contaminated food) occur each year in the U.S., if under-reporting and under-diagnosis are taken into account.

Giardiasis is more prevalent among children than among adults, possibly because many individuals seem to have a lasting immunity after infection. However, chronic, symptomatic giardiasis is more common in adults than in children. Some seasonality of infections is noted, with more cases observed in the U.S. during summer months, and might reflect increased outdoor recreation and exposure to contaminated water (e.g. lakes, swimming pools).

4. Sources

Infection typically results after ingestion of soil, water, or food contaminated with feces of infected humans or animals. Giardiasis is most frequently associated with consumption of contaminated water. However, foodborne outbreaks that were associated with vegetables and lettuce-based salads were reported in 2005 and 2007 and included 65 cases. Infected food handlers are very often implicated in giardiasis outbreaks, suggesting the ease of foodborne transmission. For example, an infected food handler preparing raw vegetables that were later served in an office cafeteria was the probable cause of nearly 30 cases. *Giardia* cysts are not killed by chlorine levels typically used to rinse produce post-harvest, and are especially difficult to wash off of complex food surfaces like leafy greens and berries.

5. Diagnosis

Giardia lamblia is frequently diagnosed by visualizing the organism, either the trophozoite (active reproducing form), or the cyst (the resting stage that is resistant to adverse environmental conditions) in stained preparations or unstained wet mounts of liquid stool, with the aid of a microscope. *Giardia* cysts are 10 to 20 μm in length and are easily distinguished from much smaller *Cryptosporidium* oocysts. Commercial direct fluorescence antibody kits are available to stain the organism, with reported sensitivities and specificities reaching 100%. Organisms may be concentrated by sedimentation or flotation; however, these procedures reduce the number of recognizable organisms in the sample. Therefore, a single stool specimen is usually insufficient for diagnosis. Enzyme linked immunosorbant assays (ELISAs) that detect excretory secretory products of the organism, as well as cyst wall proteins, are also available. In addition, non-enzymatic immunoassays exist. When compared with microscopy, such tests have sensitivities and specificities ranging from 85% to 100%.

6. Target Populations

Giardiasis occurs throughout the population, although the prevalence is higher in children than in adults; especially in children 2 to 5 years old, in daycare, where a child-to-child passage rate as high as 50% has been noted. Studies have estimated a 15% prevalence rate in U.S. children and prevalence rate up to 30% in children from younger than 10 years old in developing countries. Adults who recreate outdoors (e.g. hunters, backpackers) and may ingest contaminated water are also particularly vulnerable, due to increased exposure to *Giardia* in the environment. Other high-risk groups include individuals with certain antibody deficiencies and those with decreased gastric acidity.

7. Food Analysis

Food is analyzed by thorough surface cleaning of the suspected food and sedimentation of the organisms by centrifugation of wash material. A fluorescent antibody staining technique is then used to identify *Giardia* cysts.

8. Examples of Outbreaks

- For more information on recent outbreaks see the Morbidity and Mortality Weekly Reports from the Centers for Disease Control and Prevention (CDC).
- CDC provides an annual listing of foodborne disease outbreaks in the U.S.

9. Other Resources

- Loci index for genome *Giardia lamblia*
- GenBank Taxonomy database
- CDC *Giardiasis* FAQ - Frequently Asked Questions about *Giardiasis*.
- USDA FSIS Parasites and Foodborne Illness Resource page
- FDA Bacteriological Analytical Manual: Current recovery methods are published in this FDA methodology reference. The FDA continues to actively develop and improve methods of recovering parasitic protozoa and helminth eggs from foods.

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Entamoeba histolytica

1. Organism

Entamoeba are single-celled protozoan parasites capable of infecting a wide variety of hosts. All species are characterized by a life cycle that alternates between two distinct stages. The cyst stage is the infectious, but nonreplicative, form of the parasite that will develop in the intestine of the host into active trophozoites capable of replicating. Trophozoites multiply by binary fission and can also produce cysts.

Although at least eight species of *Entamoeba* can infect humans, only one species, *Entamoeba histolytica*, causes invasive disease in humans. *E. histolytica* is morphologically identical to non-pathogenic *E. moshkovskii* and *E. dispar.* Presently, the genome sequences of seven *E. histolytica* strains and one from *E. dispar* are available in public databases. (See Resources section).

A rigid wall protects the cysts, which may remain viable in a moist environment for weeks to months. The cysts can survive freezing and are not always killed by chlorination; however, they do not survive desiccation or temperatures above 50°C.

2. Disease

Entamoeba histolytica causes amebiasis (or amoebiasis).

- **Mortality**: According to the World Health Organization (WHO), amebiasis is the third leading cause of death due to parasitic disease globally. The WHO estimates that approximately 50 million people worldwide suffer from invasive amebic infection each year, resulting in 40,000 to 100,000 deaths annually. The global case fatality rate is reported to be 2% in adults and

For Consumers: A Snapshot

Entamoeba (pronounced entaMEEbuh) is a parasite that can make people sick. It's not very common in the U.S., where it usually affects people who traveled here from a country with poor sanitation. People who have a weak immune system or take medicines that lower the actions of the immune system (such as some drugs for rheumatoid arthritis or cancer) are more at risk of illness than are otherwise healthy people. *Entamoeba* is passed in the bowel movements of infected people and can spread to others. For example, food can become contaminated if it's handled by an infected person who didn't wash his or her hands well after a bowel movement, or if contaminated water is used for growing fruits or vegetables or to rinse them afterwards. It doesn't take many *Entamoeba* cells to cause illness, and tiny bits of a bowel movement can pass from the unwashed hands of an infected person (even if they look clean) onto the hands and into the mouth of another person, causing that person to become infected. It may also be possible to become infected from swimming-pool and other water meant for recreation. In most cases, people have no symptoms and might not know they're infected, but they can still pass *Entamoeba* in their bowel movements. When symptoms do occur, they range from mild diarrhea to severe diarrhea that contains mucus and blood, and a swollen abdomen. Sometimes the illness becomes long-lasting or permanent, with weight loss and tiredness. In rare cases, it can spread to other parts of the body. If it goes to the liver, it can also cause fever, pain, and tenderness in the upper right part of the abdomen, and nausea. The spread of *Entamoeba* can be prevented through good personal hygiene; for example, washing your hands well after bowel movements and by following other food-safety tips – including cooking, since temperatures above 122°F kill *Entamoeba*. (But be aware, when cleaning, that chlorine – bleach – might not kill *Entamoeba*.)

26% in children. In the United States, cases of *Entamoeba histolytica* infection are not common, and mortality is likely to be rare.

- **Infective dose:** The infective dose in humans is reported to be fewer than 10 cysts.

- **Onset**: Invasive intestinal disease may occur days to years after initial infection; however, the condition generally will be manifested within 2 to 4 weeks after first exposure to this parasite.

- **Illness / complications:** About 10% of *E. histolytica* infections result in clinical symptoms. Intestinal amebiasis manifests mostly as asymptomatic colonization, in which the parasite lives within the digestive system, but does not penetrate intestinal cells. Most infected people eliminate the parasite from the gut within 12 months. In some people, the disease will progress into amoebic colitis after invasion of the intestinal mucosa. On rare occasions (2% to 20% of symptomatic infections), the disease will spread extra-intestinally, mostly to the liver, causing amebic liver abscess, or to the brain, spleen, lungs, or genitourinary tract. The disseminated forms of the disease are associated with higher mortality rates.

  Given the small, but substantial, risk of invasive disease and the potential to transmit infection to others, the WHO recommends treating all cases of proven *E. histolytica*, regardless of symptoms. Different regimens are available, depending on the disease's stage of progression.

- **Symptoms**: When symptoms do occur, they usually begin within months after amoebas first enter the body. The severity of the symptoms associated with intestinal amebiasis ranges from mild diarrhea to a severe, dysentery-like illness with mucus and blood in the diarrhea and abdominal distention. A chronic infection often leads to weight loss and fatigue. Amoebic liver abscess is characterized by fever, pain in the upper right abdomen, nausea, unintentional weight loss, and liver tenderness.

- **Duration**: *E. histolytica* may reside in the intestine for years without causing symptoms. Invasion of the intestine will cause symptoms that can last from a few days to several weeks, in the absence of treatment. Treatment may be necessary to prevent recurrent attacks.

- **Route of entry:** The primary mode of infection is the fecal-oral route. Both cysts and trophozoites are passed in the feces, but trophozoites do not survive gastric acid.

- **Pathway**: Epidemiologic observations suggest that genetic differences within the host or the parasite itself may determine the outcome of clinical infection; however, what triggers the invasive phenotype in *E. histolytica* has not been elucidated. To become invasive, trophozoites secrete toxins that break down the intestinal protective mucus layer, destroy the colonic intestinal barrier, and counter the defense mechanisms of the host.

3. Frequency

The global prevalence of infection was estimated, in 1986, to be 10% of the world's population. However, this estimate was made prior to the separation of *E. histolytica* and non-virulent

E. dispar, which seems to be more prevalent worldwide. Most infections, morbidity, and mortality occur in South and Central America, Africa, and Asia (Far East and Indian subcontinent). In the U.S., the combined prevalence of *E. histolytica / E. dispar* is estimated to be 4%.

4. Sources

As noted in the Organism section, above, cysts have several characteristics conducive to survival in the environment. Once they are excreted into the environment, fecal contamination can result in *E. histolytica* cysts in drinking water, foods, hands, surfaces, and other objects. Water is the most common source of contamination. Raw foods also may be a source of infection, after contamination by a food handler or by irrigation / rinse water, especially if the food is maintained in a moist environment.

Humans are the only hosts for *E. histolytica*. People who have chronic amebiasis or are asymptomatic can excrete several million cysts per day. During the acute phase of the illness, people tend to shed more trophozoites than cysts.

5. Diagnosis

Clinically, it is desirable to distinguish pathogenic *E. histolytica* from non-pathogenic *E. dispar* and *E. moshkovskii*. Light microscopic examination of fecal specimens for cysts and trophozoites does not allow for such differentiation, unless red blood cells are identified inside trophozoites, a strong indication of invasive amebiasis. Biopsy, serology, antigen detection and molecular assays can be used for the specific diagnosis of *E. histolytica*; however, some of these technologies are less accessible to areas of the world where amebiasis is endemic.

6. Target Populations

Infection with *E. histolytica* is endemic in many parts of the world where sanitation is poor. Children are among the most affected. In industrialized countries, this infection is most common among immigrants from endemic areas, travelers to developing nations, and in institutionalized populations. Males are more prone to develop amebic liver abscess than are females.

7. Food Analysis

The FDA Bacteriological Analytical Manual describes use of successive rinses and sedimentation steps in detergent solutions to recover protozoa from vegetables (Chapter 19; Section V; see Resources section). However, the procedure is not very sensitive, as less than 1% of the initial parasitic population may be recovered. In addition, the cysts may be too damaged for efficient microscopic diagnosis.

8. Examples of Outbreaks

In developed countries, amebic infections tend to cluster in households, in institutions housing people with developmental delayed, or among sexual partners. Large outbreaks remain rare. The largest outbreak in the U.S. occurred during the 1933 World's Fair, in Chicago, and involved sewage contamination of drinking water, leading to about 1,400 cases and 98 deaths. More recently, an outbreak of amebiasis was reported in the Republic of Georgia, with 177 cases recorded between May 26 and September 3, 1998, including 71 cases of intestinal amebiasis and 106 probable cases of liver abscess. Water contamination was suspected, but not confirmed.

9. Resources

- Centers for Disease Control and Prevention information about *Amoeba histolytica*
- *Entamoeba* home page of the London School of Hygiene and Tropical Medicine
- AmoebaDB, an amoeba genomics resource
- FDA's Bacteriological Analysis Manual chapter on parasitic animals in food

Recent reviews:

Weedall GD, Hall N. Evolutionary genomics of *Entamoeba*. Res. Microbiol. 2011 Jul-Aug;162(6)637-645.

Lejeune M, Rybicka JM, Chadee K. Recent discoveries in the pathogenesis and immune response toward *Entamoeba histolytica*. Future Microbiol. 2009Feb;4(1)105-18.

Pritt BS, Clark CG. Amebiasis. Mayo Clin Proc. 2008 Oct;83(10)1154-18.

Ali IK, Clark CG, Petri WA Jr. Molecular epidemiology of amebiasis. Infect Genet Evol. 2008Sep;8(5)698-707.

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Foodborne Pathogenic Microorganisms and Natural Toxins

Cryptosporidium parvum

1. Organism

Cryptosporidium parvum is an obligate, intracellular protozoan parasite first recognized as a human pathogen in 1976. The organism is transmitted via oocysts (i.e., the infectious stage in the organism's life cycle) and shed in feces.

Among C. *parvum's* notable characteristics is the oocyst's pronounced resistance to most chemical disinfectants, including chlorine, although it is susceptible to drying and the ultraviolet portion of sunlight. Even after a 90-minute contact time with standard concentrations of chlorine-containing compounds, the reduction in levels of viable organisms is barely appreciable.

Other notable characteristics are *C. parvum*'s particular risk to, and often poor or fatal outcome among, immunocompromised people, including those with HIV/AIDS, and the amount of fluid loss the infection can cause through diarrhea.

A number of other *Cryptosporidium* species (*C. canis*, *C. felis*, *C. meleagridis*, and *C. muris*) can infect humans; however, such infections are rare and usually are detected and/or isolated from immunocompromised persons or children. Outside of humans, *C. parvum* has also been isolated from cattle, sheep, and goats. This broader host range translates into more opportunities for pathogen spread and occurrence in the environment. *C. hominis* resembles *C. parvum* in appearance and life cycle characteristics, but infects only humans.

For Consumers: A Snapshot

Cryptosporidium can cause extremely large amounts of diarrhea when it contaminates food, drinks, or "play" water, including swimming pool water or other outdoor water, if it's swallowed. The amount of body fluid lost from this illness can be dangerous for anyone, **but people who have a weak immune system, such as those with HIV/AIDS or cancer, are at especially high risk.** In these people, the diarrhea can become even more severe or last a long time or permanently, or the infection can spread to the liver and lungs, for example. They are at risk of dying from the infection. Otherwise healthy people who get this illness usually get better in 2 days to 2 weeks. For anyone who has severe or longer-lasting diarrhea, seeing a health professional is very important. This is especially important for people with a weak immune system, including people with HIV/AIDS. The illness usually starts a week or a little longer after a person eats or drinks *Cryptosporidium* in food or water. Beside large amounts of watery diarrhea, symptoms might include nausea, vomiting, cramps, and fever. The life stage of *Cryptosporidium* that causes infection is called the oocyst. The oocysts can spread when they're passed in the bowel movements of infected people or animals and end up on hands, food, water, or other objects. Bowel movements can contain the oocysts for months after symptoms are gone. The spread of *Cryptosporidium* can be prevented by washing fresh fruits and vegetables and through good personal hygiene; for example, washing your hands well before handling food and after going to the bathroom and by following other basic food-safety tips. Oocysts are very resistant to chlorine (for example, bleach used for cleaning) and may not be killed, but can be inactivated by boiling in water for several minutes.

Typically, human exposure occurs via ingestion of water contaminated with fecal material from an infected animal or food that was irrigated or washed with contaminated water.

2. Disease

The disease caused by *Cryptosporidium* is cryptosporidiosis.

- **Mortality**: Death from cryptosporidiosis is very rare. However, immunocompromised people have increased morbidity and mortality associated with cryptosporidiosis.

- **Infective dose:** As few as 10 to 100 oocysts. Oocysts are excreted in a fully infective form.

- **Onset**: Onset of illness follows an incubation period of 7 to 10 days.

- **Illness / complications:** Intestinal cryptosporidiosis is self-limiting in most otherwise healthy people. Some infected people are asymptomatic; in others, symptoms may range from mild to profuse diarrhea, with passage of 3 to 6 liters of watery stool per day. In some outbreaks involving day-care centers, diarrhea has lasted from a week to a month. Dehydration is a major concern, particularly for pregnant women and young children and immunocompromised people in whom the infection becomes chronic.

  Immune status has a strong influence on the severity and duration of symptoms and illness. In people with AIDS and in other immunocompromised people, *C. parvum* infections are notorious for their severe symptoms and outcomes, including chronic and/or copious diarrhea and dehydration, and may lead to death. This population may have the disease for life, and the major fluid loss they experience may contribute to their death. Among people with concurrent HIV infection, the CD4+ count can help predict the severity of cryptosporidiosis, according to the Centers for Disease Control and Prevention (CDC). A level less than 180 cells/mm^3 appears to be a trigger point; above that level, cryptosporidiosis usually self-resolves.

  Extraintestinal forms of cryptosporidiosis exist, with biliary cryptosporidiosis being the most common type. Other forms involve the lungs and middle ear. People with AIDS are more susceptible than are others to extraintestinal cryptosporidiosis.

  Treatment

  Preventing dehydration is critical, given the large amount of fluid loss typical of this illness. To date, there is no known drug that is effective as a treatment for cryptosporidiosis. Some relief from diarrhea has been noted with administration of spiramycin given near the onset of infection. The FDA has approved nitazoxanide for the treatment of diarrhea in immunocompetent people. A limited number of studies also have reported value in administering azithromycin, nitazoxanide, and paromomycin to *Cryptosporidium*-infected people with AIDS.

- **Symptoms**: The most common symptom is profuse, watery diarrhea, along with nausea, vomiting, and cramping. Fever can also accompany these symptoms. As noted, the severity and duration of diarrhea usually is increased in immunodeficient people, in whom the diarrhea may become chronic and who are more susceptible to extraintestinal symptoms.

- **Duration**: Two to 14 days in immunocompetent people; often prolonged or chronic in immunocompromised people. It is important to note that excretion of oocysts can last for up to several months after diarrhea has resolved. In addition, high percentages of people without overt symptoms have been found to shed oocysts. This is of major concern, since people who appear healthy may be transmitting the illness through inadequate hygiene or even through use of recreational-water facilities – especially since one bowel movement may result in the release of up to 10^9 oocysts.

- **Route of entry**: Oral.

- **Pathway**: Cryptosporidiosis is acquired through ingestion of the oocyst, the organism's infective stage. The oocyst is 4 to 6 μm in diameter, about half the size of a red blood cell. After being ingested, *C. parvum* oocysts attach themselves to gastrointestinal epithelial cells, where reproduction takes place. The zygotes become one of two types of sporulated oocysts: one with a thin wall, which excysts in the gastrointestinal tract and can cause continued infection of the host, and the other with a thick wall, which is shed in the feces and infects other hosts.

  The mechanism by which the organism causes illness – e.g., whether or not a toxin is present – is not fully understood. The mechanisms underlying extraintestinal cryptosporidiosis also are unclear; however, it is believed that the intestines are the originating site.

3. Frequency

Direct human surveys indicate a prevalence of about 2% of the population in North America. Serological surveys indicate that 80% of the United States population has had cryptosporidiosis at some point in life. Data from the Centers for Disease Control and Prevention (CDC), collected from 2004 to 2007, indicate an increase in cryptosporidiosis across the U.S. Estimates by the CDC, updated in 2011, place the number of annual illnesses due to cryptosporidiosis to be in the tens of thousands, much higher than the 7,500 that are laboratory-confirmed. The extent of illness associated with reactive sera is not known.

4. Sources

Food

Cryptosporidium spp. contamination could occur, theoretically, with any food touched by an infected food handler or from contact with an environmental source of oocysts (e.g. animal manure). In addition to various foods, such as fresh produce, juices and milk may be contaminated.

Water

Large outbreaks also have been associated with contaminated water supplies. Irrigation water might be a potential source of food contamination, even if the water is chlorine-treated.

Recreational water, such as swimming-pool water, continues to be a major vehicle for transmission of *Cryptosporidium* oocysts. The oocysts are notoriously hard to inactivate with disinfectants, like chlorine, and can remain infectious for up to a year in both freshwater and

seawater. Treated human wastewater can contain oocysts and could contaminate recreational waters, as can direct contamination by a person with poor hygienic practices.

5. Diagnosis

Because *Cryptosporidium* oocysts are shed in the infected person's feces, stool samples are collected and analyzed with a combination of light microscopy and acid-fast staining. Care needs to be taken, as oocysts can be confused with yeast cells or mistaken for *Cyclospora* cysts or even completely overlooked due to their small size. Commercially available kits use highly specific fluorescent antibodies to stain and positively identify the organisms isolated from feces as *Cryptosporidium* spp. Diagnosis also has been made by staining the trophozoites in intestinal and biopsy specimens. Pulmonary and tracheal cryptosporidiosis is diagnosed by biopsy and staining. Molecular-based tests (i.e., PCR) have also been developed and successfully implemented in some laboratories.

6. Target Populations

Cryptosporidiosis can affect anyone; however, as noted, the most severe symptoms occur in immunocompromised people. Those with AIDS seem to be highly susceptible, with the possibility of developing a chronic state of illness and an extraintestinal manifestation of disease.

People at increased risk of cryptosporidiosis include those who share a household with an infected person, health-care workers, day-care personnel, users of recreational waters, and those traveling to endemic areas. Child day-care centers serve a large, susceptible population and frequently report outbreaks. Incidence of disease is higher in child day-care centers that serve food, compared with those that do not.

7. Food Analysis

FDA's Bacteriological Analytical Manual includes a method for examination of fresh produce and liquids (milk, juice, water, cider) for *Cryptosporidium* spp.

8. Examples of Outbreaks

See CDC's cryptosporidiosis surveillance, U.S., 2006-2008.

A number of recognized foodborne outbreaks of cryptosporidiosis occurred in the U.S. in the 1990s. Some of the most notable include:

- Minnesota (chicken salad)
- Maine and New York (apple cider)
- Washington (unknown food)
- In October 2003, locally produced (Northeast Ohio) ozonated apple cider was linked to an outbreak of cryptosporidiosis.

For more information on recent outbreaks see CDC's Morbidity and Mortality Weekly Reports.

9. Other Resources

- Loci index for genome *Cryptosporidium parvum*
- GenBank Taxonomy Database
- FAQ – CDC Fact Sheet: Cryptosporidiosis. Includes Epidemiology and Risk Factors, Disease, Diagnosis, Treatment, Control, Prevention, Surveillance, Outbreak Management, and Information for Special Groups and Settings
- Emerging Infectious Diseases 13(1) 2007

  Subtypes of *Cryptosporidium parvum* in Humans and Disease Risk
- Emerging Infectious Diseases 12(4) 2006

  Cryptosporidiosis Associated with Ozonated Apple Cider
- Emerging Infectious Diseases 1(2)1995

  Waterborne Cryptosporidiosis Threat Addressed
- Emerging Infectious Diseases 3(1)1997

  Cryptosporidiosis: An Emerging, Highly Infectious Threat
- Emerging Infectious Diseases 3(4)1997

  Genetic Polymorphism Among *Cryptosporidium parvum* isolates: Evidence of Two Distinct Human Transmission Cycles
- USDA FSIS Parasites and Foodborne Illness Resources Page – *Cryptosporidium parvum*
- 10. Molecular Structural Data:
- Fayer R, Xiao L. *Cryptosporidium* and cryptosporidiosis
- Ortega Pierres MG, Cacciò S, Fayer R, Mank TG, Smith HV, Thompson RCA. *Giardia* and *Cryptosporidium*: from molecules to disease

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Cyclospora cayetanensis

1. Organism

Cyclospora are single-celled protozoan parasites and are classified as obligate intracellular coccidian parasites in the phylum *Apicomplexa*. Species of *Cyclospora* develop in the gastrointestinal tract of vertebrates throughout their entire live cycle. Immature (unsporulated) oocysts are then shed in feces. Though there are many species of *Cyclospora*, only *Cyclospora cayetanensis* has been observed to cause illness in humans.

For Consumers: A Snapshot

If eaten, fresh produce contaminated with *Cyclospora* can cause illness, although this is very rare in the U.S. It's more common in areas around the tropics, and people who travel to those areas are at higher risk of getting this illness. Some people have no symptoms, but in others, symptoms begin about a week after eating the contaminated food. They include diarrhea (sometimes explosive), loss of appetite, weight loss, cramps, bloating, nausea, and tiredness. More severe cases may include flu-like symptoms, such as headache, vomiting, fever, and aching. The elderly or very young and people with weak immune systems are at higher risk of severe illness than are other people. Without treatment, symptoms can go on for days or months, or may go away and come back. Infected people pass undeveloped *Cyclospora* in their bowel movements. A couple of weeks after being passed into the outside world, the parasite becomes fully developed and can cause infection if a person eats a fruit or vegetable contaminated with it. Raspberries, lettuce, and other fresh produce imported from areas around the tropics are at higher risk of being contaminated with *Cyclospora* than is fresh produce from other regions. As always, thoroughly wash fruits and vegetables to help reduce the risk of getting sick from food that may be contaminated, and follow other basic food-safety tips, too.

2. Disease

- **Mortality**: No deaths were attributed to *Cyclospora* in a Centers for Disease Control and Prevention (CDC) estimate of deaths from foodborne illness acquired in the United States, published in January 2011.

- **Infective dose:** The minimum infective dose of oocysts, the oocyst sporulation rate, and their survival under different environmental conditions are unknown.

- **Onset**: The onset of illness from infection with *Cyclospora cayetanensis* is usually 7 to 10 days from the time of ingestion.

- **Disease / complications:** This parasite can cause protracted diarrheal illness in both immunocompetent and immunocompromised humans. Infection with *Cyclospora* oocysts and its accompanying illness is associated with eating fresh foods contaminated with feces. Immature *Cyclospora* oocysts that are shed in feces require a period of time, usually 1 to 2 weeks, outside the body (exposed to the environment) to mature and become infective. *Cyclosporiasis*, the illness caused by infection with *Cyclospora cayetanensis*, is characterized by prolonged, watery diarrhea, and intestinal distress.

- **Symptoms**: Symptoms typically include watery diarrhea, with frequent, sometimes explosive bowel movements. Other common symptoms include loss of appetite, weight loss, abdominal cramping and bloating, nausea, and fatigue. In some instances, more severe, flu-like symptoms (headache, vomiting, fever, and body aches) may be observed, while in others no overt symptoms are observed.

- **Duration**: If left untreated, symptoms may persist for days to months. Relapses are possible.

- **Route of entry**: Oral.

- **Pathway**: *Cyclospora cayetanensis* infects cells that line the small intestine.

3. Frequency

Cases of cyclosporiasis are exceedingly rare. Though several large outbreaks of food-associated illness have been documented in the late 1990s and early 2000s, sporadic individual cases and small clusters of illness rarely exceed 100 to 200 cases, per year. Strong evidence suggests that *Cyclospora* infection is seasonal. For example, epidemiologic studies indicated that, in Peru, the season was from December to July; in the U.S., May to July; and in Nepal, May to August.

4. Sources

Foods implicated in outbreaks of cyclosporiasis include imported fresh produce, such as raspberries, basil, and several varieties of lettuce.

5. Diagnosis

Identification of this parasite is made through symptoms and through microscopic examination of stool specimens. Shape and size characteristics of immature (unsporulated) oocysts present in the stool help to confirm a *Cyclospora* infection. *Cyclospora cayetanensis* oocysts are perfectly round and 8 to 12 micrometers in diameter. In addition, when viewed by ultraviolet fluorescence microscopy, *Cyclospora* oocysts have the appearance of a pale-blue halo.

6. Target Populations

People of all ages are susceptible to infection with *Cyclospora cayetanensis*. Those who live in tropical and sub-tropical regions of the world are at greater risk. Though not fully understood, disease transmission and illness appear to be seasonal and have frequently been associated with the rainy season in those affected areas. People who travel to these areas are at risk.

7. Food Analysis

Because of its size and the inability to culture *Cyclospora cayetanensis* in the laboratory, it is extremely difficult to isolate and detect this pathogen from foods, since the levels of contamination are usually low. Currently food rinses are analyzed by microscopic examination and by molecular biological methods, such as PCR. Key points for laboratory analysis are included in:

- FDA method (eBAM 19a): *Cyclospora* and *Cryptosporidium* (2004)
  Detection of *Cyclospora* and *Cryptosporidium* from Fresh Produce: Isolation and Identification by Polymerase Chain Reaction (PCR) and Microscopic Analysis.
- FDA Laboratory Information Bulletin 4044 (1996).
  Differentiation of *Cyclospora* sp. and *Eimeria* spp. by Using the Polymerase Chain Reaction Amplification Products and Restriction Fragment Length Polymorphisms.

8. Examples of Outbreaks

For examples of outbreaks, see CDC's Morbidity and Mortality Weekly Reports.

9. Other Resources

- Loci index for genome Cyclospora cayetanensis
- Information from CDC.
- USDA FSIS Parasites and Foodborne Illness Resource page.

Bad Bug Book

Foodborne Pathogenic Microorganisms and Natural Toxins

Trichinella species

1. Organism

Trichinella spp. are parasitic roundworms (nematodes). The larvae of these worms, which reside in animal skeletal muscle, infect other animals or humans that consume them. Among several *Trichinella* species and genotypes, *Trichinella spiralis* found in domestic and wild pigs causes the most human illness worldwide. Other *Trichinella* species generally found in animals other than domestic pigs, particularly in wild game, also cause human illness. These include *T. murrelli*, *T. nativa*, *T. pseudospiralis*, *and Trichella genotype T6* in North America.

2. Parasite Life Cycle

First-stage worm larvae, about 1 mm long, may lie dormant for years in the host's skeletal muscle, waiting to be consumed by a new animal or human host. Upon ingestion by a new host, the larvae activate and invade the epithelium of the small intestine. After four molts over 1 to 2 days, male and female worms become sexually mature and find each other in the small intestine to mate. About 5 days after they were consumed as first-stage larvae, fertilized adult females begin shedding newborn first-stage larvae in the host's intestine. Each female releases thousands of newborn larvae that leave the intestine and migrate throughout the host's body via the lymph and blood circulatory systems. The migrating larvae can invade most host tissues, including heart, brain, eye, lung, and liver; however, they survive only in skeletal muscle, where they settle and become infective to the next host.

For Consumers: A Snapshot

These kinds of worms can infect people who eat undercooked meat, especially from wild game, such as bear and wild boar. Domestic pigs also can carry the worms, although modern farming practices have reduced the number of infected pigs in the U.S. At first, the worms might cause no symptoms or mild symptoms, like diarrhea and abdominal discomfort, and sometimes nausea and vomiting. In the bowel, the worms mature and produce more larvae (an immature stage of the worm), which travel to other parts of the body, through the circulation; for example, to the liver, muscles, eyes, etc. These larvae usually cause symptoms a week to a month later; for example, muscle pain, fever, weakness, and, often, swelling around the eyes. But the larvae can survive only in certain muscles (muscles that attach to bone), where they can live for years, often without causing further symptoms. The larvae in the other parts of the body, such as the liver, die, which can cause inflammation and other problems in those body parts. It's rare for people to die from these worms, but it sometimes happens when the dead larvae cause problems. If the disease can be detected in the early stage, treatment can reduce the number of larvae produced in the bowel. But since there usually are no symptoms or only mild symptoms in the early stage, the disease often isn't detected until later, after the larvae travel through the body. At this later stage, medication can reduce inflammation and the possibility of other problems. The best idea: Don't get these worms, in the first place – follow the U.S. Department of Agriculture's recommendations on how to cook wild game and other types of meat, to make them safe to eat.

3. Disease

Trichinellosis, or trichinosis, is the name of the disease caused by *Trichinella* larvae consumed in undercooked meat.

- **Mortality**: Mortality is rare; roughly 0.2 percent of clinical cases worldwide result in death.

- **Infective dose**: The ingestion of two viable larvae (male and female) that successfully mature and mate can result in infection with thousands of newborn larvae; however, ingestion of several larvae are normally needed to enable successful reproduction and noticeable symptoms. A statistical analysis of disease outbreak data estimated that ingestion of 5 larvae resulted in a mean 1% chance of observable disease symptoms; ingestion of 10 larvae resulted in a 7.5% chance; and ingestion of 100 larvae resulted in a 45% chance.

- **Onset**: The acute symptoms of trichinellosis, which correspond with the migration of newborn larvae, typically begin 1 to 4 weeks following the consumption of contaminated meat, depending on the severity of the case. The initial intestinal infection may cause earlier mild gastrointestinal symptoms, particularly if many larvae are consumed.

- **Symptoms:**

    - The duration and severity of symptoms are variable, depending on such factors as how the meat was prepared, the number of larvae ingested, the *Trichinella* species, and the immune status of the patient.

    - Symptoms associated with the initial invasion of the intestinal epithelium by ingested larvae are usually mild or not apparent, but may include diarrhea, abdominal discomfort, and, possibly, nausea and vomiting.

    - The acute and sometimes severe symptoms of trichinellosis, which are associated with newborn larval invasion of body tissues, commonly include muscle pain, fever, and weakness. Facial swelling, particularly around the eyes, is often present.

- **Complications**: While larvae survive only in skeletal muscle, complications can develop, because larvae migrate through, and may perish in, various other organs. Invasion of heart, brain, or lung tissue, and subsequent inflammation, sometimes leads to death. Complications usually develop within the first 2 weeks of infection and are more common with heavy infections.

- **Duration**: Within a few weeks, larvae-producing females are inactivated and eliminated from the intestine by the patient's immune system. The acute symptoms associated with newborn larval migration gradually decline over the course of a few weeks, as newborn larvae become dormant in skeletal muscle. Patients remain asymptomatic while *Trichinella* larvae live in their muscles for years; however muscle pain, fever, and other symptoms can sometimes linger.

- **Route of entry**: Oral ingestion of live larvae in undercooked meat. The disease is not transferred from person to person.

4. Diagnosis and Treatment

Early symptoms, if present, resemble common gastrointestinal illness, and the patient usually does not seek treatment. Diagnosis of the later, acute-phase symptoms is difficult, because their onset is often weeks after the undercooked meat was eaten, and the symptoms are varied and may be similar to other diseases, such as the flu. Confirmation of trichinellosis is based on the presence of typical symptoms, combined with laboratory test results, and, sometimes, evidence of consumption of parasitized meat. Infected patients usually have an elevated eosinophil blood cell count. Immunological tests (ELISA, Western blot) are used to detect the presence of *Trichinella*-specific antibodies in the patient's blood serum; however, these antibodies take 2 or more weeks to develop. Sometimes a muscle biopsy for the presence of larvae is performed. If larvae are recovered from a food source or patient, the exact species can be determined using molecular methods.

Timely treatment usually includes anthelmintic drugs to remove any remaining fertile adult worms from the intestine. Normal anthelmintic doses generally do not kill the newborn larvae migrating in tissues, nor is this desirable, because of the possibility of a hazardous inflammatory response to dead larvae in sensitive organs. Therefore, early diagnosis and treatment is needed in order for anthelmintics to effectively reduce the severity of disease by shortening the period of newborn larval production. Glucocorticosteroid drugs may be used to reduce inflammatory and allergic immune responses that may cause complications and to reduce pain. Anthelmintic drugs are always used with early glucocorticosteroid administration, because the steroid may disrupt the immune system's normal ability to remove fertile worms from the intestine. Severely infected or pregnant persons should be hospitalized during acute symptoms, to ensure prompt treatment of any complications.

5. Sources

Infective *Trichinella* larvae occur in undercooked meats from carnivorous and omnivorous mammals, reptiles, and birds. The most important source worldwide is the domestic pig; however, in the U.S. and other developed countries, the risk of infection from domestic pigs has been dramatically reduced. Pork now causes less than half of U.S. *Trichinella* infections, while meats other than pork, particularly from game animals, cause the most illness. More than 150 mammalian species are known to harbor *Trichinella* larvae, including horses, boars, bears, cattle, walruses, seals, cougars, deer, badgers, beavers, raccoons, foxes, dogs, skunks, squirrels, cats, and rats. Undercooked bear meat has caused several illnesses in North America.

6. Target populations

Target populations include hunters and other consumers of undercooked game, people who eat undercooked pork attained from a questionable source, and people who eat traditional uncooked meat dishes, such as walrus and horse meat.

7. Frequency

Between 2000 and 2007, an average of 13 laboratory-confirmed illnesses were reported, per year, through the U.S. National Notifiable Disease Surveillance System. However, adjusting for under-diagnosis and under-reporting, the frequency of domestically acquired trichinellosis is

estimated to be between 40 and 340 illnesses per year. Much higher infection rates *per capita* occur in less-developed pork-consuming nations and in countries that have disruptions in modernized pork production due to political upheavals.

8. Prevention

Trichinellosis is prevented by thoroughly cooking meat. See Section 3-401 of the FDA Food Code for details on safe cooking times and temperatures for meats.

Ready-to-eat pork products distributed in the U.S. must be processed in a manner that inactivates *Trichinella spiralis*, or the source carcass must be inspected for the presence of *Trichinella* larvae. Pork sold in grocery stores is intended to be cooked by the consumer and is not inspected for *Trichinella*. However, preventive controls implemented at commercial pig farms have reduced *Trichinella*-contaminated pork in the U.S. to negligible levels. These controls include cooking meat byproducts fed to pigs and preventing rodents from entering pig enclosures. A small chance still exists to attain commercial pork from a domestic or foreign farm using poor pig-husbandry practices; therefore, the careful consumer should continue to thoroughly cook pork to inactivate *Trichinella* and other potential pathogens.

Commercial deep-freezing processes are sometimes used to deactivate *Trichinella* larvae in ready-to-eat pork products, in which the principle species of concern is *T. spiralis*. Freezing is not an appropriate preventive control to use at home or to use with non-pork animals; freezing-resistant species of *Trichinella* are found in non-pork and game meats. *Trichinella* larvae have been shown to survive in frozen bear meat for 5 years.

9. Food Analysis

Carcasses may be directly inspected for *Trichinella* larvae using an artificial digestion method. A pepsin and acid mixture is used to dissolve the meat and leave the digestion-resistant larvae, which are then concentrated and enumerated under a microscope. A sample usually consists of 1 gram of meat from a location in the carcass that is ordinarily most infected with larvae. This method does not ensure that the carcass is free from infection, but with proper usage it will consistently detect at least one larva when there are more than three larvae per gram of meat. Carcasses used for ready-to-eat pork products that do not undergo any other USDA-approved *Trichinella* deactivating process must be inspected using a more sensitive 5-gram sample. Consistently negative inspection results provide powerful statistical evidence that the source area or herd is *Trichinella*-free.

Tests to detect *Trichinella* antibodies in animal blood serum are sometimes used to survey pig herds, wild animal populations surrounding pig production facilities, and wild-game populations. The sensitivity of serology testing is lower for light infections, and serology does not detect recently parasitized animals that have not yet developed sufficient antibodies, a process that takes 2 to 9 weeks.

10. Examples of Outbreaks

In May 1988, an Alaskan woman and her sister-in-law became ill with trichinellosis. They had consumed dried walrus meat, whale blubber, beaver, and ducks. A walrus had been killed and divided among residents of two villages. Of the 51 persons who ate the walrus meat, 27 became ill with case-defined trichinellosis.

In January 1995, a hunter shot a cougar in Idaho and made cougar jerky by salting and smoking the meat. A couple of weeks later, he became ill with trichinellosis symptoms. The jerky and cougar were found to contain *Trichinella nativa* and *Trichinella* genotype T6 larvae. The hunter had distributed the jerky to 14 other persons, 9 of whom were also found to have case-defined trichinellosis.

In August 2000, four hunters from Wisconsin killed a black bear in Alaska and fried and ate the meat the same day. Two to four weeks later, they all came down with typical acute trichinellosis symptoms. The bear meat was analyzed and found to contain 24 *Trichinella* larvae per gram.

11. Other resources

Centers for Disease Control and Prevention (CDC), Trichinellosis

CDC, Morbidity and Mortality Weekly Report

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Pozio E. 2007. World distribution of *Trichinella* spp. infections in animals and humans. Vet. Parasitol. 149:3-21

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Foodborne Pathogenic Microorganisms and Natural Toxins

Taenia species

1. Organism

The pork tapeworm (*Taenia solium*), the beef tapeworm (*Taenia saginata*), and the Asian tapeworm (*Taenia asiatica*) are flatworm parasites in the class Cestoda that mature in the human small intestine.

Parasite Life Cycle

These tapeworms require a mammalian intermediate host and a human final host to complete their life cycles. Worm segments (proglottids) filled with mature eggs separate from adult tapeworms and pass with human feces, or, in the case of *T. saginata*, the proglottids can crawl out on their own. The eggs can survive for months on the ground or in water. Pigs and cattle will consume human feces and may ingest *Taenia* eggs with feces or with food and water. After pigs ingest *T. solium* or *T. asiaticia* eggs, or cows ingest *T. saginata* eggs, the oncosphere larvae hatch, penetrate the gut, and disseminate to various tissues via the bloodstream. After encysting in tissue, the larvae develop into bladder-like cysticerci, about 1 cm in size, that live for many months. In humans, *T. solium* larvae encyst in tissue, just as in the pig intermediate host; however, the cysticerci in human tissue cannot complete the worm's life cycle. To complete the worm's life cycle, humans must ingest live cysticerci in undercooked pork or beef. Ingested cysticerci activate and attach to the intestinal wall, where they grow 2 to 7 meters long and produce eggs for many years.

For Consumers: A Snapshot

People can become infected with these kinds of tapeworms by eating undercooked pork or beef. The beef tapeworm also can cause infection when the worm's eggs are on a person's fingers or in soil or food, for example, and the eggs get into the person's mouth and end up in his or her bowel. These worms can attach to the inside of the bowel, grow to about 6 to 20 feet long, and live there for years. During that time, the eggs are passed into the environment and eaten by pigs, cattle, or humans, continuing the cycle of infection. It may be months or longer before people even know they're infected. With intestinal infections, many people have no symptoms; others might have diarrhea, nausea, pain, and change in appetite, and may just not feel well. Medication can get rid of the worms in these cases. But the beef tapeworm can cause very serious problems if the eggs are swallowed and the larvae they become at an early stage of the worm's development end up in the brain or other vital organs. If the worm is in the brain, delicate surgery is required that can be dangerous. To keep from being infected with these worms, cook roasts and steaks until the center of the thickest part reaches at least 145°F for at least 3 minutes, wash your hands well before eating and after handling soil, and follow other food-safety tips, like washing vegetables well before eating them. (Note: Cook ground meat to at least 160°F in the center, to also kill bacteria).

2. Disease

The disease caused by intestinal infection with adult *Taenia* tapeworms is **taeniasis**. The disease is similar among the three species. However, infection with *T. solium* is more serious than the others, because of the risk of **cysticercosis**, the disease caused by tissue infection with *T. solium*

larval cysts (cysticerci). Neurocysticercosis, in which cysticerci lodge in brain tissue, is the severe form of cysticercosis.

- **Mortality**:
    - Taeniasis: None known.
    - Cysticercosis: Death occurs in a small percentage of neurocysticercosis cases.
- **Infective dose**:
    - Taeniasis: Ingestion of one live cysticercus larva in meat or viscera of pork or beef can result in infection with an intestinal tapeworm.
    - Cysticercosis: Ingestion of one *T. solium* egg may result in a tissue infection with one cysticercus larva. Ingestion of multiple eggs increases the likelihood of serious illness.
- **Onset**:
    - Taeniasis: Tapeworms mature in the intestine and begin to release eggs about 2 to 4 months after live cysticerci are ingested.
    - Cysticercosis: Cysticerci larvae reach full size in tissues about 2 months after *T. solium* eggs are ingested. Neurocysticercosis symptoms may be delayed for several years.
- **Symptoms**:
    - Taeniasis: Usually asymptomatic, but may cause abdominal pain, nausea, diarrhea, change in appetite, and general malaise.
    - Cysticercosis: Cysts located in the brain may be asymptomatic or can present multiple symptoms depending on the number, location, size, and condition of cysts, and on the immune response of the patient. Common neurocysticercosis symptoms include seizures, increased intracranial pressure, headache, and altered mental status. Cysts in the eye usually affect vision. Muscular and subcutaneous cysts may be felt, but do not cause much pain.
- **Complications**:
    - Taeniasis: Complications are unlikely. *T. solium* proglottids rarely may rupture while in the small intestine, releasing eggs that may auto-infect the individual with cysticercosis.
    - Rarely, motile *T. saginata* proglottids block the biliary duct, pancreatic duct, or appendix.
    - Cysticercosis: Live cysticerci can mask themselves from the immune system, resulting in few symptoms. However, when cysticerci degenerate, either naturally or from drug treatment, the immune system reacts, and severe inflammation can result. Cysts in brain tissue (parenchyma) are associated with seizures and are the

most common cause of epilepsy in countries in which *Taenia* is endemic. Cysts that lodge in brain-fluid spaces (ventricles, subarachnoid space) may cause blockage of cerebrospinal fluid leading to hydrocephalus (water on the brain), inflammation of the meninges, or other complications. Cysts in the brain's fluid spaces can grow very large (10 to 20 cm). Cysts in the eye can cause permanent vision damage.

- **Duration**:
    - Taeniasis: Adult worms can live for years in the intestine, but can be effectively eliminated with anti-*Taenia* drugs.
    - Cysticercosis: Cysticerci live for months, but remain in tissue for years. Neurological symptoms may appear several years after infection, and gradually decline with degradation and elimination of the cysticerci. Calcareous remnants in brain parenchyma are associated with recurring seizures years later.
- **Route of entry**: Oral
- **Pathway**:
    - Taeniasis: Ingested cysticerci in insufficiently cooked pork and beef activate and attach directly to the wall of the small intestine using suckers and/or hooks on the head (scolex).
    - Cysticercosis: Eggs released in feces of pork tapeworm carriers (humans) contaminate the environment. Persons become infected when contaminated food, fingers, or soil enter the mouth. Oncosphere larvae hatch, penetrate the intestine, disseminate in the bloodstream, and encyst to become cysticerci in the central nervous system, musculature, or visceral organs.

3. Target populations

Taeniasis occurs in populations that consume raw or undercooked beef and pork, particularly in regions with poor sanitary facilities and where, out of economic necessity, livestock are allowed to roam freely. In the United States, the disease occurs primarily in individuals who have traveled or immigrated from endemic regions in Latin America, India, Asia, Eastern Europe, and Africa.

Cysticercosis occurs where there is a high incidence of taeniasis caused by the pork tapeworm (*T. solium*). Persons infected with an adult *T. solium*, or living with an infected person, are particularly likely to accidentally ingest eggs and get cysticercosis.

4. Sources and prevention

Taeniasis: The primary source is infected beef and pork. Governments and farmers can reduce the occurrence of contaminated meats by implementing sanitary toilet facilities, controlled animal roaming, and meat inspection. Individuals can prevent taeniasis and other diseases by thoroughly cooking beef and pork product. In roasts and steaks, the center of the thickest part should measure at least 145° F for at least 3 minutes. Ground meat should reach 160° F because of the increased bacterial pathogen hazard.

Cysticercosis: An adult *T. solium* pork tapeworm in the human intestine can produce hundreds of thousand of eggs, per day, that can survive for months under harsh environmental conditions. Eggs contaminate surfaces and food when toilet or handwashing facilities are inadequate or improperly used; for example, when food handlers have poor personal hygiene practices. Risk of cysticercosis is reduced by frequent handwashing, by keeping fingers and other objects away from the mouth, and by avoiding questionable food-service establishments in endemic areas, such as where humans and pigs live in close proximity under poor sanitary conditions. A healthy individual should be tested for *Taenia* infection if a family member or other close contact is found to have cysticercosis or taeniasis.

5. Frequency

These worms infect tens of millions of people worldwide. Taeniasis and cysticercosis are not nationally reportable diseases in the U.S. *T. saginata* appears to cause the most cases of taeniasis, but is less hazardous than *T. solium*, because it does not cause cysticercosis. In recent years, *T. solium* cysticerci have not been found in commercial pork in the United States. *T. saginata* cysticerci are still occasionally found in cattle. Between 1985 and 1994, *T. saginata* cysticerci were found in 1 to 2 beef carcasses per 10,000 inspected. Most cases of taeniasis occurring in the U.S. are attributed to travel or immigration from endemic areas, mostly in Latin America. *T. solium* eggs, introduced by tapeworm carriers traveling from endemic regions, continue to cause cysticercosis in the U.S.

Nine percent of a cross-section of the El Paso, Texas, population tested positive for taeniasis in 2004, and several cases of neurocysticercosis have been reported in that city. Between 1991 and 2008, about 7,000 Los Angeles County residents were discharged from hospitals after treatment for cysticercosis (including repeated hospitalizations for the same infection). About 4,000 of these hopitalizations were for neurocysticercosis. According to a survey of death certificates, about 221 cysticercosis-related deaths occurred in the United States from 1990 to 2002.

6. Diagnosis and Treatment

Taeniasis: Diagnosis is difficult because the disease is usually asymptomatic. Detection often occurs when the patient sees proglottids in stool or undergarments. Traditionally a stool specimen is examined microscopically for characteristic *Taenia* eggs; however, this test is not reliable. A more sensitive fecal ELISA test has good diagnostic results in research settings. Anthelmintic drugs effectively eliminate adult tapeworms. The tapeworm species can sometimes be determined by examination of expelled proglottids and/or the scolex. Molecular sequencing, where available, should be used to determine the exact species.

Cysticercosis: Symptoms usually occur a long time after infection, leading to difficulty relating symptoms to the cause; because the disease is uncommon in the U.S., misdiagnosis can occur. Computed tomography and magnetic resonance imaging are needed to identify the number, location, size, and other important characteristics of cysticerci in the brain. Less expensive serological techniques are constantly improving and may be used to confirm diagnosis and track the course of disease. Cysts in the eye are viewed with an ophthalmoscope. Cysts under the skin can be felt and may be biopsied. Treatment of neurocysticercosis varies with specific circumstances. Seizures, inflammation, and hydrocephalus must first be stabilized before considering treatment for the parasite. Treating cysticerci in the brain with anthelmintic drugs may activate a severe immune response, causing excessive inflammation; therefore, anti-inflammatory and anthelmintic drugs are used judicially to facilitate removal of cysts. Shunts

may be inserted to relieve intracranial pressure, and open or endoscopic surgery may be used to remove cysticerci in accessible locations. Recovered larvae should be identified for epidemiological reasons, because other tapeworm species also may cause cysticercosis.

7. Food Analysis

Regulatory authorities inspect pork and beef carcasses for “measled” meat by feeling and visually examining susceptible muscles for cysticerci. Animals may also be tested by more sensitive serological methods.

8. Examples of Outbreaks

Taeniasis and cysticercosis are endemic in much of the world. Outbreaks may go unrecognized until complications manifest. In 1978, there was a large cysticercosis outbreak in West New Guinea after infected pigs were introduced into that country. The disease was recognized when there was an epidemic of burn victims who sustained their injuries during neurocysticercosis-induced seizures that occurred as they slept near fireplaces. In 1990, a neurocysticercosis outbreak occurred in an Orthodox Jewish community, in New York City, that did not eat pork. The source of infection was thought to be recently immigrated housekeepers infected with pork tapeworms.

9. Other resources

CDC, Taeniasis

CDC, Cysticercosis

CDC, Morbidity and Mortality Weekly Report

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Anisakis simplex and related worms

1. Organisms

Larvae of some nematodes (roundworms) in the family anisakidae can infect humans who eat raw or undercooked fish or cephalopods (marine mollusks, such as squid, octopus, and cuttlefish). These worms include:

- *Anisakis simplex* complex (herring worm)
- *Pseudoterranova (Phocanema, Terranova) decipiens* complex (cod or seal worm)
- *Anisakis physeteris*
- *Contracaecum* species

A. simplex has been responsible for the majority of human infections, with most of the rest due to *P. decipiens*. These two species are now known to be complexes of multiple species that are distinguishable only by genetic analysis. These worms average 2 to 3 cm in length.

For Consumers: A Snapshot

These worms are common in fish and squid, cuttlefish, and octopus, but proper cooking (described below) inactivates them. If you eat them alive in raw or undercooked fish, they can infect your stomach or intestine. Sometimes, the only symptom is tickling caused by a worm crawling up the throat. If a worm burrows into the wall of the stomach or intestine, it can cause stomach or abdominal pain, nausea, vomiting, and diarrhea, from mild to severe. Sometimes these worms cause an allergic reaction. Symptoms of the infection start 24 hours to 2 weeks after the fish is eaten. (The infection might be mistaken for other illnesses, so if you develop symptoms after eating seafood, be sure to tell your doctor what you ate.) The worm can live for only about 3 weeks in humans; then it dies and is eliminated, although the pain may last longer. The worm generally is found and removed with an instrument called an endoscope; if done early, the symptoms usually go away immediately. A better idea is to prevent the infection in the first place. You can help protect yourself by following the FDA Food Code guidelines for cooking fish; that is, cook fish until the inside is at 145°F for at least 15 seconds, at 155°F for fishcakes, and at 165°F for stuffed fish. Note: some people may have the allergic reaction even from cooked seafood.

2. Disease

The name of the disease caused by these worms is anisakiasis or anisakidosis.

- **Mortality:** None known.
- **Infective dose**: One worm.
- **Onset**: Symptoms usually occur within 24 hours after consumption of affected raw or undercooked fish, but may be delayed by as long as 2 weeks.
- **Symptoms / complications:** Non-invasive anisakiasis is often asymptomatic or sometimes is diagnosed when the affected person feels a tingling sensation in the throat and coughs up or manually extracts a nematode.

  Invasive anisakiasis occurs when a worm burrows into, and attaches to, the wall of the stomach or intestine. The ulceration results in an inflammatory response in which

eosinophils (white blood cells) respond and a granuloma (nodule) forms at the point of worm attachment. The symptoms may include severe stomach or abdominal pain, nausea, vomiting, and diarrhea. Symptoms may be mild, or may be characterized by a mild to strong allergic response. Occasionally, inflammation disrupts normal intestinal flow, leading to constipation. Rarely, worms penetrate through the digestive tract and are found in the body cavity.

Some people have allergic reactions when consuming dead *Anisakis* remnants in cooked or previously frozen fish, and some fish handlers have reportedly become hypersensitive to touching infected fish.

- **Duration of symptoms**: Unless complications develop, anisakiasis is a self-limiting disease in humans. Marine mammals are the worms' natural final host. Humans are an accidental host, and, in humans, the worm dies and is eliminated spontaneously from the lumen of the digestive tract within about 3 weeks. However, pain associated with inflamed lesions may occasionally persist for weeks to months after the worm has died. Symptoms usually clear immediately if the worm is removed early.

- **Route of entry**: Oral.

- **Pathway**: Burrowing in gastrointestinal mucosa.

3. Diagnosis and Treatment

In cases in which the patient vomits or coughs up a worm, the disease may be diagnosed by morphological examination of the nematode.

The symptoms of invasive anisakiasis may be misdiagnosed as appendicitis, Crohn's disease, gastric ulcer, gastrointestinal cancer, and other gastrointestinal diseases. Thus, a history of having eaten raw or undercooked fish is potentially an important diagnostic clue.

An endoscopic fiber-optic device, preferably, is used to visually diagnose and remove worms attached in the stomach and small intestine. In severe cases that cannot be diagnosed and treated endoscopically, abdominal surgery may be performed. Microscopic examination is used to identify a recovered nematode to the genus or "species complex" level, while molecular methods can be used to determine the exact species.

Elevated eosinophil counts (eosinophilia) may be detected during the early inflammatory response. Radiology also has been used as a diagnostic aid. Diagnostic tests for antibodies in human blood serum have been developed; however, antibodies may not yet be present or may be present from a previous infection, and some tests may cross-react with other parasites, such as *Ascaris lumbricoides*.

Treatment may include steroids, antibiotics, and isotonic glucose solution. Anthelmintic drugs are not generally considered appropriate, but have been used with some success. The worm will die and pass naturally, but endoscopic removal is considered the best treatment for severe pain.

4. Frequency

The frequency in the United States is unknown, because the disease is not reportable and can go undetected or be mistaken for other illnesses. Anisakiasis was first recognized in the 1960s.

During the 1970s, about 10 cases per year were reported in the literature. The frequency is probably much higher, due to home preparation of raw or undercooked fish dishes. In Japan, more than 1,000 cases are reported annually.

5. Sources and Prevention

These larval worms may be found in the viscera and/or flesh of almost all ocean fish and cephalopods, and occur frequently in cod, haddock, fluke, Pacific salmon, herring, flounder, monkfish, and squid. Fish and cephalopods consumed raw or undercooked, whether marinated, pickled, cold-smoked, or braised, pose a risk of infection.

The FDA Food Code guidelines for cooking fish should suffice to inactivate these worms in fish and cephalopods. The guidelines for fish are as follows: cook the fish to an internal temperature of 145ºF for 15 seconds; to 155ºF for comminuted fish, such as fish cakes, and 165ºF for stuffed fish. Commercial processors and retailers may use a specific deep-freeze process to kill parasites in fish products that are served without thorough cooking. The food and fishery industries may obtain detailed information about freezing methods for killing seafood parasites in the current edition of the FDA Fish and Fishery Hazards and Controls Guidance.

6. Food Analysis

Candling (examination of fish on a light table) is used by commercial processors to reduce the number of visible nematodes in certain white-fleshed fish known to be infected frequently. This method is not totally effective, nor is it very adequate to remove even the majority of nematodes from fish with pigmented flesh.

Pepsin digestion is used in scientific studies to dissolve fish tissue while leaving pathogenic parasites intact. Because this method is time-consuming, it is generally not used for routine food analysis.

7. Examples of Outbreaks

This disease is known primarily from individual cases. Japan, where a large volume of raw fish is consumed, has the greatest number of reported cases.

8. Other Resources

- Centers for Disease Control and Prevention, Division of Parasitic Diseases, DPDx: Anisakiasis
- National Center for Biotechnology Information, Taxonomy Database: Anisakidae
- FDA guidance on controlling parasite hazards for seafood processors: FDA Fish and Fishery Hazards and Controls Guidance, Fourth Edition, chapter 5.
- Lymbery AJ, Cheah FY. Anisakid nematodes and anisakiasis. In: Murrell KD, Fried B, eds. *World Class Parasites: Volume11, Food-Borne Parasitic Zoonoses, Fish and Plant-Borne Parasites*. New York, NY: Springer Science; 2007:185-207

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Diphyllobothrium species

1. Organisms

Diphyllobothrium latum and about 13 other flatworms of the genus *Diphyllobothrium* are intestinal parasites of humans and other fish-eating mammals and birds. They are also called "broad tapeworms" and "fish tapeworms."

For Consumers: A Snapshot

Eating certain raw or undercooked fish, even if it's salted, marinated, or cold-smoked, can cause humans to become infected with tapeworms. Tasting the ingredients of a fish dish before cooking it also can cause infection with tapeworms. People sometimes don't know they're infected with these worms, which can grow up to 32 feet long and live for 25 years in humans. Symptoms usually are mild abdominal discomfort, diarrhea, and changes in appetite, and may begin in about 10 days. After some time, pieces of the worm might be seen in bowel movements. The worm absorbs a large amount of vitamin B12 from the human intestine. Without enough of this vitamin, humans don't make enough healthy red blood cells and may develop vitamin B12-deficiency anemia. Heavy infection with many tapeworms may block the bowel. The worm is easily killed with medications prescribed by a health professional. You can help protect yourself against tapeworms by following the FDA Food Code guidelines for cooking fish; that is, cook fish until the inside is at 145°F for at least 15 seconds, at 155°F for fishcakes, and at 165°F for stuffed fish.

2. Disease

The disease caused by this organism, diphyllobothriasis, results from consumption of *Diphyllobothrium* spp. larvae, which are found in the meat and viscera of raw or undercooked fresh fish. After consumption, the larvae attach in the small intestine and grow rapidly. Eggs begin to be produced and expelled in the patient's stool as early as 15 days after consumption of the larvae. Adult tapeworms grow up to 32 feet (about 10 meters) long and can produce about a million eggs per day.

- **Mortality**: None known.
- **Route of entry**: Oral.
- **Infective dose:** One or more larval worms.
- **Onset:** The tapeworm produces eggs as early as 15 days after consumption; however, the infection usually is not noticed.
- **Symptoms / complications:** Infection with *Diphyllobothrium* usually presents no noticeable symptoms, or the symptoms are mild, including abdominal discomfort, diarrhea, and altered appetite. The tapeworm absorbs a great amount of vitamin B12, which, in prolonged or heavy cases, may cause a vitamin B12 deficiency that rarely leads to anemia. Intestinal obstruction has been known to occur in rare massive infections.

3. Diagnosis and Treatment

Patients often become initially aware of an infection by observing pieces of the tapeworm in their stools. Diagnosis is made by demonstration of the characteristic eggs during microscopic examination of a stool sample. The eggs are easily confused with similarly shaped parasitic

trematode eggs. Molecular methods may be used to identify *Diphyllobothrium* to the species level. Worms can survive in the small intestine for more than 25 years, but are easily expelled with drugs (praziquantel and niclosamide) when the worms are discovered.

4. Frequency

Diphyllobothriasis is considered a minor public health problem, and records are no longer maintained on the frequency of the disease. From 1977 to 1981, in the United States, 100 to 200 cases were reported, per year. The actual number of cases was probably much higher, considering asymptomatic and mild cases that went unreported. An estimated 20 million people currently are infected, worldwide.

5. Sources and Prevention

Human infection with *Diphyllobothrium* is caused by eating raw or undercooked fish dishes (including those that have been marinated, salted, or cold-smoked); e.g., sushi, sashimi, ceviche, and tartare. Tasting ingredients of fish dishes before they are cooked (e.g., gefilte fish) also can cause infection. Infective larvae are found in the meat and viscera (i.e., eggs, liver) of freshwater and marine finfish from temperate latitudes. In North America, these fish include Pacific salmon and freshwater fish, such as pike, perch, walleye, burbot, char, Alaska blackfish, dolly varden, whitefish, and trout. Imported, fresh fish from temperate climates also may contain infective larvae.

The FDA Food Code guidelines for cooking fish should suffice to inactivate these worms. The guidelines for fish are as follows: cook the fish to an internal temperature of 145ºF for 15 seconds; to 155ºF for comminuted fish, such as fish cakes, and 165ºF for stuffed fish. Commercial processors and retailers may use a specific deep-freeze process to kill parasites in fish products that are served without thorough cooking. The food and fishery industries may obtain detailed information about freezing methods for killing seafood parasites in the current edition of the FDA Fish and Fishery Hazards and Controls Guidance.

6. Target Populations

Any consumer of raw or undercooked fish.

7. Food Analysis

Foods are not routinely analyzed. Microscopic inspection of thin slices of fish flesh, or artificial digestion of the flesh, can be used to detect the "plerocercoid" larvae.

8. Examples of Outbreaks

An outbreak involving four Los Angeles physicians occurred in 1980. These physicians all consumed sushi (a raw fish dish) made of tuna, red snapper, and salmon. Others who did not consume the sushi made with salmon did not contract diphyllobothriasis. In 1980, the CDC determined that 19 of 25 diphyllobothriasis cases in the Los Angeles area likely resulted from consuming salmon. A few individual cases in foreign countries have been attributed to the consumption of Pacific salmon originating in North America.

9. Other Resources

- CDC, Division of Parasitic Diseases, DPDx: Diphyllobothriasis
- Information on outbreaks: CDC, Morbidity and Mortality Weekly Reports
- National Center for Biotechnology Information, Taxonomy Database: *Diphyllobothrium* spp.
- FDA guidance on controlling parasite hazards for seafood processors: FDA Fish and Fishery Hazards and Controls Guidance, Fourth Edition, chapter 5.

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Nanophyetus salmincola

1. Organism

Nanophyetus salmincola is a small parasitic trematode (fluke) in the flatworm phylum.

2. Disease

Nanophyetiasis is the name of the human disease caused by these intestinal flukes when they are consumed live in raw or undercooked fish. At least one newspaper report has referred to the disease as "fish flu."

These worms also are known to carry a bacterium that causes a serious, sometimes fatal disease in dogs (salmon poisoning disease); however, this bacterium is not known to infect humans.

- **Mortality**: None known, in humans.
- **Infective dose:** Approximately 500 worms are required to elicit symptoms.
- **Route of entry**: Oral. Ingestion of worm larvae (metacercariae) encysted in fish flesh or viscera; also by hand-to-mouth contact while handling infected fish.
- **Onset**: Eggs can be detected in stool about 1 week after a contaminated fish is ingested.
- **Symptoms**: Patient complaints include abdominal pain, diarrhea, gas / bloating, and nausea / vomiting. Seven of 20 reported cases in the United States were asymptomatic. Increased numbers of circulating eosinophils (>500/µl) were found in 50% of the cases.
- **Duration:** Without treatment, symptoms may last several months.

For Consumers: A Snapshot

Eating fish that have lived in certain waters (described below) can transmit this worm and cause illness, unless the fish are properly cooked. The worms usually cause mild symptoms or none. (A bacterium in the worms can infect and kill dogs if they aren't treated; however, in humans, it's the worm itself, not the bacterium, that causes illness.) In the U.S., only about 23 people are known to have gotten infected with the worms, but the number could be higher. Some people might not know they have the worm or may think they have some other illness. Raw or undercooked salmon and other fish that spend time in freshwater streams in the Northwestern U.S. and British Columbia can transmit the worm. Even hand-to-mouth contact can transmit it to people who handle heavily contaminated raw or undercooked fish. About a week after a person eats contaminated fish, the worm's eggs start to appear in the person's bowel movements. Symptoms may include abdominal discomfort, diarrhea, nausea, and vomiting. Without treatment, symptoms may last several months, but medications prescribed by health professionals kill the worms. A better idea is to prevent the infection in the first place. You can help protect yourself by following the FDA Food Code guidelines for cooking fish; that is, cook fish until the inside is at 145°F for at least 15 seconds, at 155°F for fishcakes, and at 165°F for stuffed fish.

3. Parasite Life Cycle

N. salmincola eggs released by adult worms hatch as miracidium larvae in rivers and streams. Miracidium larvae penetrate a pleurocerid stream snail (first intermediate host) and undergo

asexual replication. Cercariae larvae are shed by the snail and penetrate the skin of a fish (secondary intermediate host), where they encyst as metacercariae larvae in the fish flesh and viscera. The final hosts are fish-eating mammals and birds. When a mammal (including humans) consumes an infected fish, the larvae attach and mature in the small intestine.

4. Target populations

Target populations include consumers of raw or undercooked (including home-smoked) fish from the sources discussed below.

5. Sources and prevention

Fresh fish originating in, or passing through, coastal streams of Oregon, Washington, northern California, southeast Alaska, and British Columbia, where the intermediate snail host lives, are sources of infection with this worm. Salmonids (e.g., salmon, trout, steelhead) are more heavily infected with larval worms. Fish from areas of eastern Siberia and Brazil that have appropriate pleurocerid snail intermediate hosts may also contain the worm. In anadromous fish (fish that migrate from freshwater streams / lakes to the ocean and return), the infective cysts survive the period spent at sea. Aquacultured salmonids fed only pelleted feed could be infected if the fry / smolts originated from hatcheries with water sources that contain N. *salmincola* cercariae.

The FDA Food Code guidelines for cooking fish should suffice to inactivate these worms. The guidelines for fish are as follows: cook the fish to an internal temperature of 145ºF for 15 seconds; to 155ºF for comminuted fish, such as fish cakes, and 165ºF for stuffed fish. Commercial processors and retailers may use a specific deep-freeze process to kill parasites in fish products that are served without thorough cooking. The food and fishery industries may obtain detailed information about freezing methods for killing seafood parasites in the current edition of the FDA's Fish and Fishery Hazards and Controls Guidance.

6. Frequency

In the U.S., 20 of the 23 known cases were in patients of a single Oregon clinic. Because symptoms are mild or absent, many cases probably are not identified. Two cases occurred in New Orleans, well outside the endemic area, reflecting the likelihood of interstate commerce of commercial fish containing the parasite. In some villages in eastern Siberia, more than 90% of the human population is infected with this worm.

7. Diagnosis

Differential diagnosis is indicated by gastrointestinal symptoms and a history of eating fresh raw or undercooked salmonids from endemic areas. Definitive diagnosis is made by detecting the worm's characteristic eggs in the patient's stool. The eggs are difficult to distinguish from those of *Diphyllobothrium latum;* however, the treatment for both infections is the same.

8. Treatment

Nanophyetiasis is a mild illness, and the worms will pass naturally, if the practice of eating undercooked fish is stopped. Treatment with anthelmintic drugs (e.g., praziquantel) clears the symptoms and stops egg production.

9. Food Analysis

There are no established methods for detection of *Nanophyetus salmincola* cysts in fish flesh. The cysts are small (0.5 mm long by 0.25 mm wide). Candling with the aid of a dissecting microscope, or pepsin HCl digestion, should detect heavily infected fish. A homogenation-sedimentation technique has been used, with reported success.

10. Examples of Outbreaks

There have been no major outbreaks.

11. Other Resources

Adams AM, DeVlieger DD. Seafood parasites: prevention, inspection, and HACCP. In: Hui YH, Sattar SA, Murrell KD, Nip WK, Stanfield PS, eds. *Foodborne Disease Handbook, Vol. 2, 2nd ed*. New York: Marcel Dekker, Inc. 2001:407-423.

Eastburn RL *et al*. Human intestinal infection with *Nanophyetus salmincola* from salmonid fishes. *Am J. Trop. Med. Hyg.*1987; 36:586-591.

Fritsche TR *et al*. Praziquantel for treatment of human *Nanophyetus salmincola* (*Troglotrema salmincola*) infection. *The Journal of Infectious Diseases*. 1989; 160:896-899.

Harrell LW *et al.* Human nanophyetiasis: transmission by handling naturally infected coho salmon (*Oncorhynchus kisutch*). *The Journal of Infectious Diseases*. 1990; 161:146-148.

National Center for Biotechnology Information, Taxonomy Database: Digenea

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Eustrongylides species

1. Organism

Larval *Eustrongylides* spp. are large red roundworms (nematodes) that are ½ to 4½ inches (15 to 115 millimeters) long. The larvae are found in fish.

2. Disease

The disease (eustrongylidiasis) is caused by these worms when contaminated live or raw fish are consumed and the larval nematode penetrates the wall of the human intestine.

- **Mortality**: None known
- **Infective dose:** One live larval worm can cause an infection.
- **Route of entry**: Oral.
- **Onset**: Symptoms develop within 24 hours after a contaminated live or raw fish is eaten.
- **Symptoms:** In the five cases reported, penetration of the worm into the gut wall was accompanied by severe abdominal pain.

For Consumers: A Snapshot

Five cases of infection with this worm, which humans can get by eating raw or undercooked fish, are known to have occurred in the U.S. Four were in fishermen who ate live minnows, one of many kinds of freshwater or saltwater fish that can carry the worm. In humans, the worms can cause severe pain within 24 hours after being eaten, as they work their way into the bowel wall. Surgery was done to diagnose four cases and remove the worm. In one case, a patient recovered in 4 days without surgery. There may be some risk of infection of the sterile area that holds the bowel, if worms break through the bowel wall and into the sterile area that holds the bowel and infect that area with bowel bacteria. The risk of getting these worms from sushi is reduced by the U.S. requirement that fish used for sushi undergo a freezing process (which can't be achieved by most home freezers) to kill worms. When you cook fish, you can help protect yourself by following FDA Food Code guidelines; that is, cook fish until the inside is at 145°F for at least 15 seconds, at 155°F for fishcakes, and at 165°F for stuffed fish.

- **Complications:** The abdominal pain is similar to appendicitis, and four of the five reported cases required investigative surgery. During surgery, worms were found in the peritoneal cavity or in the process of penetrating the gut wall. Intestinal damage and inflammation can occur during gut penetration, and other tissues could be damaged during any subsequent larval migration. The disease has the potential to cause bacterial infection of the peritoneal cavity from intestinal contents or the worm itself.
- **Duration of symptoms**: Unknown. The symptoms were resolved by surgery. In one suspected case in which surgery was not performed, the symptoms resolved in 4 days.

3. Parasite Life Cycle

Adult *Eustrongylides* spp. live in the gastrointestinal tract of fish-eating birds, such as herons, egrets, and mergansers (the definitive hosts). The parasite's eggs pass with bird feces into the

water. The eggs may be eaten by, and the larvae develop in, an oligochaete worm that lives in fresh or brackish water (an intermediate host). Fish become infected with parasite larvae by eating contaminated oligochaete worms, contaminated smaller fish, or directly from consumption of the parasite's eggs. Parasite larvae encyst in the fish's viscera and/or muscle. Birds become infected by eating contaminated fish, worms, or other intermediate hosts (amphibians and reptiles also have been reported as intermediate hosts). Humans may ingest live larvae with raw or undercooked fish. While the parasite cannot complete its life cycle in humans, it may attach to, and penetrate, the wall of the human digestive tract.

4. Target populations

The target populations are consumers of raw or undercooked fish that have not been previously frozen to kill parasites. Four of the five cases reported resulted from fishermen swallowing live, whole minnows used for bait.

5. Sources and prevention

Eustrongylides larvae are found in the flesh and viscera of a wide variety of fish from fresh, brackish, or salt waters. Whole minnows (i.e., that still contain the viscera) from estuaries may be a significant source, because their viscera frequently contain the larvae. Fish-eating bird populations near fresh or brackish water have the highest prevalence of the adult parasites; therefore, nearby fish, or fish that feed on fish that pass through such areas, are more likely to be contaminated. For example, fish raised in freshwater ponds with numerous fish-eating birds present may contain greater numbers of these worms.

The FDA Food Code guidelines for cooking fish should suffice to inactivate these worms. The guidelines for fish are as follows: cook the fish to an internal temperature of 145ºF for 15 seconds; to 155ºF for comminuted fish, such as fish cakes, and 165ºF for stuffed fish. Commercial processors and retailers may use a specific deep-freeze process to kill parasites in fish products that are served without thorough cooking. The food and fishery industries may obtain detailed information about freezing methods for killing seafood parasites in the current edition of the FDA Fish and Fishery Hazards and Controls Guidance.

6. Frequency

Extremely rare; only five cases reported.

7. Diagnosis

The illness is not fully diagnosed until the worm is identified after surgery. The abdominal pain that occurs is similar to the symptoms of appendicitis; however, parasitic worm infection may be suspected if the patient has recently eaten raw or incompletely cooked fish. Endoscopic, non-surgical discovery and removal of the worm also may be possible.

8. Food Analysis

These large red worms may be seen without magnification in fish flesh and are normally very active after death of the fish. The larva is similar in appearance to that of the kidney worm (*Dioctophyma renale*).

(The giant kidney worm – Dioctophyma renale – *is a close relative of* Eustrongylides *that normally matures in the right kidney of fish-eating mink and other fish-eating mammals. The kidney worm is a potential human health hazard in raw or undercooked freshwater fish from endemic areas. To date, no human cases have been reported in the U.S.)*

9. Examples of Outbreaks

There have been no major outbreaks in the U.S.

10. Resources

- Guerin PF *et al*. Intestinal perforation caused by larval *Eustrongylides*. *Morbidity and Mortality Weekly Report*, v.31, p.383-389, 1982.
- National Center for Biotechnology Information, Taxonomy Database: *Eustrongylides*

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Selected Amebas Not Linked to Food or Gastrointestinal Illness:

Balamuthia mandrillaris, Naegleria fowleri, Acanthamoeba species

1. Organisms

The amebas included in this section, *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Acanthamoeba* species, are not known to cause gastrointestinal illnesses or to be transmitted by food. They should not be confused with the amoeba *Entamoeba histolytica* (described in a separate chapter of this book), which is transmitted by food and water and causes "amoebic dysentery." However, although not related to food or gastrointestinal illness, these other amebas can cause other serious or fatal illnesses and are included in this document because the FDA receives inquiries about them. They are ubiquitous in the environment, including in soil, water, and air.

For Consumers: A Snapshot

Three kinds of amebas (a type of single-celled organism) that *don't* cause foodborne illness are included in this book because FDA gets questions about them, and they can cause other kinds of serious or fatal illness. Even though all the amebas in this chapter are common in soil, freshwater (such as ponds, rivers, and lakes), and air, the first two illnesses described here are rare.

- One mainly affects people with weak immune systems, children, and the elderly. The amebas that cause it enter through broken skin or the lungs and travel to the brain. The illness, granulomatous amebic encephalitis, usually ends in death. The amebas that cause it are *Balamuthia mandrillaris* and species of *Acanthamoeba*.
- Illness from another ameba, *Naegleria fowleri*, can occur in healthy people who become infected when they put their head under freshwater, such as pond water. This ameba goes up the nose and enters the brain, causing primary amebic meningoencephalitis. Patients might survive with early treatment, but otherwise die within about a week.
- A third infection, amebic keratitis, can cause blindness, which can be prevented with early treatment. It mainly affects people with eye injuries or who wear contact lenses, and is caused by *Acanthamoeba*. In the U.S., most cases from this last type of ameba are from contaminated contact-lens cleaning solution, contact-lens cases not cleaned properly, and swimming while wearing contact lenses. Cases have gone up with the popularity of contact lenses.

(Another kind of ameba, *Entamoeba histolytica*, does cause foodborne illness and is described in another chapter.)

2. Diseases

■ Granulomatous amebic encephalitis (GAE)

- caused by *Acanthamoeba* spp. and *Balamuthia mandrillaris.*

Usually associated with people who are immunocompromised in some way; however, *Balamuthia* also infects immunocompetent children and elderly people. Despite frequent human contact with these widespread amebas, they rarely cause disease. Infection may occur through

broken skin or the respiratory tract. The organisms attack the central nervous system and spread to the brain, causing granulomatous encephalitis that leads to death in several weeks to a year after the appearance of symptoms. Few patients survive.

■ **Primary amebic meningoencephalitis (PAM)**

- caused by *Naegleria fowleri*.

Usually occurs in healthy people who have immersed their heads in freshwater containing *Naegleria fowleri*. Central nervous system involvement arises from organisms that penetrate the nasal passages and enter the brain through the cribriform plate. The amebas can multiply in the tissues of the central nervous system and may be isolated from spinal fluid. The disease progresses rapidly and, if untreated, death occurs within 1 week of the onset of symptoms.

Amphotericin B can be effective in the treatment of PAM, with early diagnosis. At least five patients have recovered from PAM when treated with Amphotericin B alone or in combination with other drugs.

■ ***Acanthamoeba*** **keratitis, or amebic keratitis, (AK)**

- caused by *Acanthamoeba* spp.

Occurs in people who wear contact lenses or injure an eye. In the United States, most cases are attributed to contaminated lens-cleaning solution or poor cleaning of lens-storage cases. The ameba attaches to the cornea of the eye and spreads, causing inflammation of the cornea and severe pain. If the infection is not treated quickly, severe eye damage or blindness can occur; however, prognosis is excellent with early therapy.

3. Frequency

GAE and PAM are rare in occurrence. Since these diseases were first recognized, roughly around the third quarter of the 20th century, 300 cases of GAE and 200 cases of PAM are estimated to have occurred worldwide. The rate of PAM infection is estimated to be about one case in 2.6 million exposures to contaminated water. About 5,000 AK cases are estimated to have occurred in the U.S., with increased case frequency starting in the 1980s, likely due to the rise in contact-lens use.

4. Diagnosis of Human Illness

GAE is diagnosed by finding the characteristic amebic cysts during microscopic examination of brain-biopsy tissue.

PAM can be diagnosed by the presence of amebas in the spinal fluid.

AK may be diagnosed by microscopic examination of corneal scrapings.

In each case, amebas may be cultured and diagnosis may be confirmed by immunofluorescent and PCR techniques.

5. Food Analysis

Not applicable. Foods are not analyzed for these amebas, because foods have not been implicated in these diseases.

6. Target Populations

Immunodeficient people, especially those infected with HIV, may be at risk for opportunistic amebic infections. However, GAE, AK, and PAM have occurred in otherwise healthy people. People in whom water has entered the nose while swimming in warm-water lakes and rivers are at increased risk for PAM. Contact-lens wearers with poor lens-care practices or who swim with their contacts on are at greater risk for AK.

7. Examples of Outbreaks

Centers for Disease Control and Prevention (CDC) outbreak information: Morbidity and Mortality Weekly Reports

8. Other Resources:

CDC, Division of Parasitic Diseases, DPDx: Free-living Amebic Infections

CDC, DPD, A-Z Index: *Acanthamoeba* Infection, *Naegleria* Infection

National Center for Biotechnology Information, Taxonomy Database: *Acanthamoeba*, *Balamuthia mandrillaris*, *Naegleria fowleri*

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Ascaris lumbricoides and *Trichuris trichiura*

1. Organism

- *Ascaris lumbricoides* (common roundworm)

  (Ascaris suum is a morphologically similar worm that infects pigs and has been implicated in some human cases.)

- *Trichuris trichiura* (whipworm)

2. Disease

Ascariasis and trichuriasis are the names of the infections caused by *Ascaris lumbricoides* and *Trichuris trichiura*, respectively. Ascariasis also is known as the common roundworm infection or large roundworm infection, and trichuriasis as whipworm infection.

Eggs of these "soil-transmitted" nematodes are deposited in the feces from infected individuals and develop in warm, moist soil, becoming infective after a few weeks. The eggs stick to surfaces and may be carried to the mouth by soil-contaminated hands, other body parts, fomites (inanimate objects), or foods.

For Consumers: A Snapshot

Common roundworms and whipworms are both included in this chapter because, although they differ in some ways, they also have some things in common. The main way people become infected with them is by swallowing bits of soil containing the worms' eggs. Contaminated vegetables might contain the soil and eggs, but, most often, the soil and eggs get into people's mouths from dirty hands or from other things with soil on them. After a person swallows the eggs of the common roundworm, the eggs hatch, and the larvae pass through the intestinal wall, then into the blood and lungs (where they can cause lung problems), and end up back in the intestines, where they develop into adult worms. Whipworms instead don't go to other parts of the body, but stay in the intestines. Infection with either worm can cause symptoms ranging from none to severe, including cramps, diarrhea (sometimes bloody), and vomiting. The worms will die by themselves, but medications may be used to kill them when there are large numbers of them. You can lower your risk of getting these worms by avoiding areas where human feces are deposited on the soil and by washing your hands. Cooking kills the eggs.

Ascariasis

Ingested *Ascaris* eggs hatch in the small intestine, and the larval worms penetrate the intestinal wall and make their way to the lungs by way of the circulatory system. In the lungs, they break out of the pulmonary capillaries into the air sacs, ascend into the throat, and, finally, descend to the small intestine again, where they grow to a length of 6 to16 inches (15 to 40 cm).

Infection with one or a few *Ascaris* spp. may be asymptomatic, unless a worm is noticed when passed in the feces, or, on occasion, when a worm is crawling up into the throat and trying to exit through the mouth or nose. Heavy infections are associated with abdominal distension and pain, nausea, loss of appetite, and vomiting. The worm's lung migration may cause a self-limiting pneumonia.

Complications: Complications are correlated with the number of worms infecting the individual. Heavy aggregates of worms may cause intestinal blockage and other intestinal complications, particularly in small children. Not all larval or adult worms stay on the path that is optimal for their development; those that wander may locate in the bile or pancreatic ducts, appendix, and other sites, causing inflammation or obstruction. Worm-wandering may be stimulated by fever, some drugs, or spicy meals.

Trichuriasis

Trichuris sp. eggs hatch in the intestine, and the larvae mature directly in the intestinal epithelium, without migrating to the lungs. When mature, the tail of the worm breaks through the epithelium and protrudes into the intestinal lumen. Adult worms stay attached in one place in the intestinal caecum or colon and are 1 to 2 inches (3 to 5 cm) long, with slender heads and thickened tails.

Most trichuriasis infections are light and asymptomatic. Moderate to heavy infections result in symptoms that may include abdominal pain, diarrhea, passage of mucus and blood in the stool, nausea, vomiting, anemia, and rectal prolapse.

Chronic infection with either of these worms is thought to contribute to growth retardation and slowed mental development in malnourished children.

Diagnosis and Treatment: Both infections are diagnosed by finding the characteristic eggs in the patient's stool. *Trichuris* worms have been found in the colon during endoscopy. The larger *Ascaris* spp. are sometimes observable in the small intestine by barium X-ray, and they can be monitored in the biliary or pancreatic ducts with ultrasound.

In the absence of reinfection and complications, these illnesses are self-limiting, because the worms die naturally within 1 or 2 years. Symptomatic infections are treated effectively with anthelmintic drugs. Rarely, complications may require surgery.

3. Frequency

Humans worldwide are infected with *A. lumbricoides* and *T. trichiura*. The occurrence of eggs in domestic municipal sewage indicates that infection rates are high. A survey of U.S. state laboratory results from 1987 showed *T. trichiura* in 1.2% and *A. lumbricoides* in 0.8% of stool samples tested, although infection severity in the U.S. is usually light and asymptomatic. Infection rates are much higher worldwide and, combined, these worms infect more than a quarter of the world's population.

4. Sources

These worms release thousands of eggs, per day, that can remain infectious in soil for years. The eggs are found in contaminated soils and in insufficiently treated fertilizers made from human sewage. Although the eggs are transmitted to humans primarily through hand-to-mouth contact, they may be transmitted via raw consumption of food crops that were contaminated with insufficiently treated sewage fertilizer.

5. Target populations

Ascariasis and trichuriasis are a particular problem in areas of poor sanitation where human feces are deposited on the soil. Children up to age 10 have the highest frequency of infection. Consumers of uncooked vegetables and fruits that are fertilized with untreated sewage are at risk. Persons in close association with pigs or who consume raw crops fertilized with pig manure may also be at risk. These diseases are also associated with the practice of consuming earth (geophagy).

6. Food Analysis

Eggs of Ascaris spp. have been detected on fresh vegetables (cabbage) sampled by FDA. Methods for the detection of Ascaris spp. and *Trichuris* spp. eggs on produce are detailed in Chapter 19 (Parasitic Animals in Foods) of the FDA's Bacteriological Analytical Manual.

7. Examples of Outbreaks

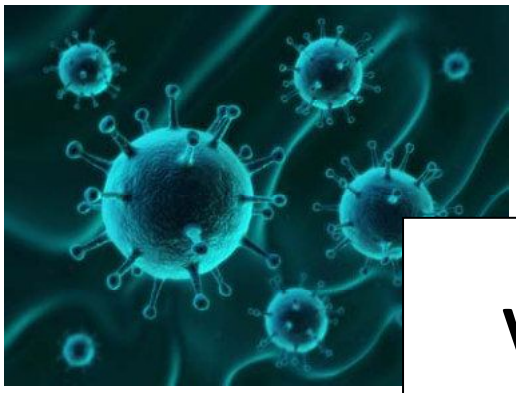
Although no major outbreaks have occurred, many individual cases occur. As noted, the illnesses are widespread.

8. Resources

CDC, Division of Parasitic Diseases, DPDx: *Ascaris lumbricoides*, *Trichuris trichiura*

Information may be found by searching CDC, Morbidity and Mortality Weekly Reports

National Center for Biotechnology Information, Taxonomy Database: *Ascaris lumbricoides*, *Trichuris trichiura*



Viruses

Noroviruses

1. Organism

Noroviruses (NoV) are environmentally hardy organisms that not only can be transmitted by food and water, but also can be easily transmitted through person-to-person contact and contact with environmental surfaces. Current concentrations of disinfectants commonly used against bacteria are not effective against these viruses.

There are five NoV genogroups (GI, GII, GIII, GIV, and GV), as determined by the RNA sequence of the virus. In 1990, the molecular cloning of the Norwalk virus genome led to the classification of this virus into the family Caliciviridae, with 29 genetic clusters within five different genogroups, and subsequently it was renamed "norovirus."

This chapter focuses on strains known to cause disease in humans, which exist primarily in genetic clusters within genogroups I, II, and IV, whereas the viruses belonging to the other genogroups have been shown to infect other animals (primarily cattle, swine, and mice).

Norovirus in genogroups GI and GII alone can be divided into at least 15 genetic clusters. A genetic cluster of NoV is defined as strains that have at least 80% homology to a reference strain's amino acid sequence.

For Consumers: A Snapshot

In the U.S., norovirus is the leading cause of illness from contaminated food or water – but food isn't the only way people get this illness. It also spreads easily from person to person and spreads quickly in groups of people. Examples of foods that have caused norovirus illness are fruits, vegetables, meats, and salads prepared or handled by an infected person. Oysters grown in contaminated water are another example. Symptoms usually start within 1 or 2 days of eating the contaminated food, but may start in as few as 12 hours. Vomiting that's explosive and projectile – that shoots out – often is the first symptom, along with watery diarrhea that isn't bloody, and cramps. Headache, mild fever, and muscle aches also may occur. Most people get better in a day or two, although it takes others a little longer. Occasionally, some people lose so much body fluid that it throws off the body's balance of some important minerals (called electrolytes) and fluid, which can cause serious health problems. These people need to be treated by a health professional, and sometimes need to be hospitalized. Antibiotics don't work against this or other viruses (they only work against bacteria), but health professionals can give the right fluids and minerals to put the body back in balance. You can help protect yourself and others against norovirus by following basic food-safety tips. Because norovirus also is spread from person to person, especially in crowded living situations – dormitories, nursing homes, day-care centers, prisons, and cruise ships are a few examples – handwashing is especially important. Norovirus spreads easily to things people touch, and other people can pick up the virus that way. It takes very little norovirus to cause illness. Although alcohol-based antibacterial hand gels work against many harmful bacteria, they don't protect against norovirus. And the virus may continue to pass in bowel movements even after symptoms have gone away – another reason to make handwashing a healthy habit.

Noroviruses constitute a genus of genetically diverse, single-stranded RNA viruses belonging to the family Caliciviridae. The NoV genome contains approximately 7.7 kilobases of genetic material protected from the environment by a naked protein capsid (i.e., no lipid-containing envelope). The icosahedral shaped capsid is composed of a major capsid protein (VP1) and a

single copy of a minor structural Protein (VP2). The 27-32 nm viral particles have a buoyant density of 1.39 to 1.40 g/ml in CsCl.

2. Disease

Common names of the illness, which is the leading cause of foodborne illness in the United States, are viral gastroenteritis, acute nonbacterial gastroenteritis, food poisoning, and winter vomiting disease.

- **Mortality**: Overall, these illnesses account for 26% of hospitalizations and 11% of deaths associated with food consumption.

- **Infective dose:** The infective dose is very low; it is estimated to be as low as 1 to 10 viral particles, and the particles are excreted at high levels by both symptomatic and asymptomatic people (as high as 1 x 10^{12} million viral particles/g feces).

- **Onset**: A mild, brief illness usually develops between 24 and 48 hours after contaminated food or water is consumed (median in outbreaks: 33 to 36 hours), but onset times within 12 hours of exposure have been reported.

- **Illness / complications:** Norovirus illness is self-limiting, but can be very debilitating as a result of the high rate of vomiting. Recovery is usually complete and without evidence of long-term effects. Dehydration is the most common complication, especially among the very young, the elderly, and patients with underlying medical conditions.

  No specific therapy exists for viral gastroenteritis, in general, or NoV infection, in particular. For most people, treatment of NoV infection is supportive; besides rest, it consists primarily of oral rehydration and, if needed, intravenous replacement of electrolytes. Currently no antiviral medication is available, and antibiotics are not effective for treating NoV infection. Presently no vaccines are available to prevent NoV infection, although this is an active area of research.

- **Symptoms:** Symptoms usually present as acute-onset vomiting (often explosive); watery, non-bloody diarrhea with abdominal cramps; and nausea. Explosive, projectile vomiting usually is the first sign of illness and is often used to characterize the illness. Headache, low-grade fever, chills, and muscle aches may also occur. The severity of symptoms appears to be higher in hospitalized patients, immunocompromised people, and elderly people, compared with younger adults and other groups.

  Studies suggest that 30% of people infected with NoV display no gastrointestinal illness or associated symptoms, but still excrete high levels of virus in their stool. These distinct groups of people are considered to be silent shedders of NoV.

- **Duration**: Symptoms generally persist for 12 to 60 hours, with a mean period of 24 to 48 hours. Most people report feeling better within 1 to 2 days. However, for hospitalized patients, immunocompromised people, and the elderly, vomiting and diarrhea generally resolve within 72 to 96 hours, while the non-specific symptoms, such as headache, thirst, and vertigo, could persist up to 19 days.

- **Route of entry**: Foodborne norovirus illnesses have been epidemiologically linked into three distinct classes: with cases associated with consumption of ready-to-eat (RTE) foods contaminated by food workers; with environmental contamination of produce; or with consumption of molluscan shellfish harvested from contaminated water. In each of these classes, transmission occurs through the fecal-oral route (or vomit, on occasion), and is often associated with improper sanitation controls or their application. Secondary transmission following foodborne illness is common, due to the high levels of virus that are excreted.

- **Pathway**: Norovirus infection causes gastroenteritis, an inflammation of the stomach and the small and large intestines. However, the precise pathogenic pathway of infection is unknown, which has hampered progress in propagating the virus in the laboratory.

3. Frequency

The Centers for Disease Control and Prevention (CDC) estimates that noroviruses cause 5.5 million illness annually in the U.S. (estimated range: 3.2 million to 8.3 million cases of foodborne illness), which accounts for 58% of all foodborne illnesses. Of these illnesses, approximately 0.03% (mean, 14,663; range, 8,097 to 23,323) require hospitalization, and less than 0.1% of these illnesses results in death (mean,149; range, 84 to 237).

4. Sources

NoV outbreaks have been associated with consumption of contaminated water, including municipal water, well water, stream water, commercial ice, lake water, and swimming pool or recreational surface-water exposure, as well as floodwater.

Salad ingredients, fruit, and oysters are the foods most often implicated in norovirus outbreaks. However, any ready-to-eat food that is that is handled by ill food workers may be contaminated. Nearly 29% of all NoV foodborne outbreaks from 1997-2004 could be attributed to food purchased or served at a restaurant or delicatessen. Molluscan shellfish, particularly oysters, have been commonly identified in NoV-related gastroenteritis outbreaks, worldwide. However, this represents a different etiology that does not necessarily involve a contaminated food worker.

The rapid spread of secondary infections is particularly evident in areas where a large population is enclosed within a static environment, such as in institutions, college campuses, schools, military operations, hotels, restaurants, recreational camps, hospitals, nursing homes, day-care facilities, and cruise ships, and after natural disasters, such as hurricanes and earthquakes.

5. Diagnosis

Clinical diagnosis, without the results of diagnostic tests used to identify NoV-associated illness, includes the following four criteria (Kaplan *et al*., 1982):

- vomiting in more than 50% of affected persons in an outbreak;
- a mean (or median) incubation period of 24 to 48 hours;
- a mean (or median) duration of illness of 12 to 60 hours;
- lack of identification of a bacterial pathogen in stool culture.

Confirmation of a clinical diagnosis of NoV infection can be achieved by performing analytical tests on serum, stool, and, in some instances, vomitus. Diagnosis also can be achieved by examining blood serum samples for a rise in virus-specific serum antibody titers, measured by enzyme immunoassay (i.e., ELISA or EIA). This analysis is premised on an increased serum titer (generally a four-fold increase) of immunoglobulins – IgA, IgG, and IgM – against the presumed viral antigen in acute or convalescent sera; however, this approach requires the collection of multiple sera samples from patients, to allow identification of an increase in sera antibodies. These have been commercially marketed to detect NoV in fecal material; however, this approach has had only a 55% level of accuracy, when compared with a reverse transcription polymerase chain reaction (RT-PCR) approach. The applicability of these assays is also limited by the requirement to collect stool specimens from acute or convalescent patients for accurate determination.

Examination of stool specimens for norovirus can be performed by microscopy (direct electron microscopy or immunoelectron microscopy), to visualize viral capsids, but requires the virus to be found at high densities (generally $>10^6$/g). Molecular techniques, such as RT-PCR, have been successfully used to detect the presence of viral nucleic acids in stool and vomitus. RT-PCR is the preferred method of diagnosis, since it is significantly more sensitive than microscopy; does not require a large, expensive electron microscope with highly skilled personnel; and has the ability to rapidly differentiate genogroups, which could be instrumental in follow-up epidemiologic investigations, to determine the route and distribution of NoV in the community.

6. Target Populations

Illness due to NoV may impact people of any age, but has been reported, through population-based studies, to be more prevalent among the elderly and children under 5 years old. Evidence suggests that there is a genetic predisposition to acquiring infection that is dependent on the patient's blood type (ABO phenotype).

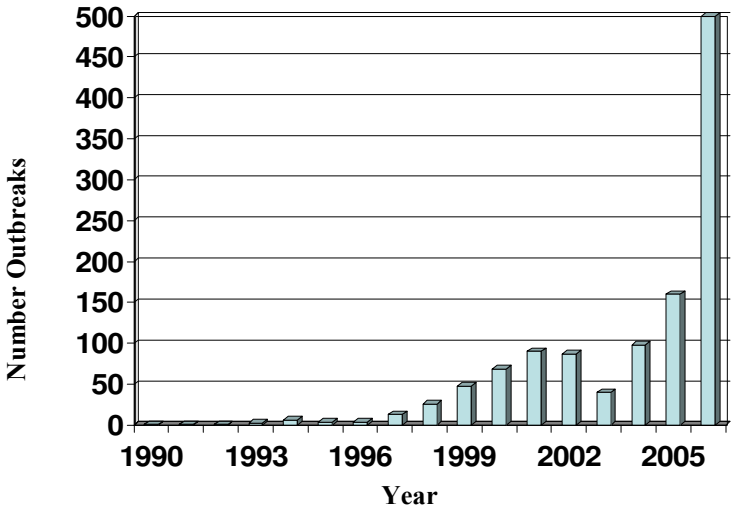
Prior infection by NoV does not provide long-term immunity, and reinfection by the same strain can occur several months after the initial infection.

The rapid spread of secondary infections is particularly evident in areas where a large population is enclosed within a static environment, such as in institutions, college campuses, schools, military operations, hotels, restaurants, recreational camps, hospitals, nursing homes, day-care facilities, and cruise ships, or after natural disasters, such as hurricanes and earthquakes.

7. Food Analysis

NoV has been successfully isolated from, and detected in, oysters, irrigation and ground water, and deli meats associated with illnesses. Quantitative RT-PCR (qRT-PCR) is the most sensitive method for NoV detection in food extracts and is an improvement over conventional RT-PCR, due to its increased specificity and sensitivity. Assays using this RT-PCR technology for NoV detection and quantitation are commercially available.

8. Examples of Outbreaks



Reported number of norovirus outbreaks in the United States from 1990 to 2007 (adapted from Centers for Disease Control and Prevention, 2008)

Selected examples of specific outbreaks from 2000-2006:

Anderson AD, Heryford AG, Sarisky JP, Higgins C, Monroe SS, Beard RS, Newport CM, Cashdollar JL, Fout GS, Robbins DE, Seys SA, Musgrave KJ, Medus C, Vinjé J, Bresee JS, Mainzer HM, Glass RI. 2003. A waterborne outbreak of Norwalk-like virus among snowmobilers – Wyoming, 2001. J. Infect. Dis. 187:303-306.

Centers for Disease Control and Prevention (CDC). 2006. Multisite outbreak of norovirus associated with a franchise restaurant -- Kent County, Michigan, May 2005. MMWR 55:395-397.

Cotterelle BC, Drougard J, Rolland M, Becamel M, Boudon S, Pinede O, Traoré K, Balay P, Pothier EE, Espié E. 2005. Outbreak of norovirus infection associated with the consumption of frozen raspberries, France. March 2005. Euro Surveill. 10(4):E050428.1.

Shieh Y, Monroe SS, Fankhauser RL, Langlois GW, Burkhardt W III, Baric RS. 2000. Detection of norwalk-like virus in shellfish implicated in illness. J. Infect. Dis. 181(Suppl 2):360–366.

9. Resources

The NCBI Taxonomy Browser contains the names of all organisms represented in the genetic databases with at least one nucleotide or protein sequence.

CDC provides a variety of information about noroviruses.

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Hepatitis A virus

For Consumers: A Snapshot

Hepatitis A is an illness caused by the hepatitis A virus. One of the ways people can become infected with HAV, although it's not the most common way, is by eating or drinking contaminated food or water. Contaminated water, shellfish, and salads are the foods most often linked to outbreaks, although other foods also have been involved in outbreaks. The illness usually is mild, starts about 2 to 4 weeks after the contaminated food or water is eaten or drunk, and goes away by itself in a week or two, although it can last up to 6 months in some people. It causes inflammation of the liver, and symptoms may include fever, low appetite, nausea, vomiting, diarrhea, muscle aches, and yellowing in the whites of the eyes and the skin (jaundice). In rare cases, the illness can quickly cause severe liver damage, leading to death. The virus spreads from the feces (bowel movements) of infected people. For example, when infected people have a bowel movement and don't wash their hands well afterwards, or when people clean an infected person who has had a bowel movement and don't wash their hands well, they can spread the virus to anything they touch, and other people can pick it up when they touch that same surface later. Day-care centers are among the places where this can easily happen. When the virus gets on the hands of people who prepare food, they can contaminate the food and spread the virus to people who eat the food. Countries with poor sanitation also are high-risk places, and travelers should be aware that some water in those countries may be contaminated. Cooking food until it's at a temperature of 190˚F in the middle for at least 1½ minutes or boiling food in water for at least 3 minutes inactivates the virus. Common cleaners aren't usually sold in the strengths needed to destroy this virus, and it can withstand more heat than many bacteria can. It can also withstand freezing. Good handwashing is one of the best things you can do to help protect yourself and others from HAV, along with other basic food-safety tips.

1. Organism

Hepatitis A virus (HAV) particles are environmentally hardy organisms that can be transmitted by contaminated food, water, environmental surfaces (e.g., contaminated table tops, cooking utensils) and through direct or indirect person-to-person contact. Although HAV cannot grow in the environment, they are considered to be extremely stable under a wide range of environmental conditions, including freezing, heat, chemicals, and desiccation. Concentrations of disinfectants commonly used against pathogenic bacteria are not considered effective against these viruses.

There are six HAV genotypes (I-VI), as determined by RNA sequence analysis at the VP1-2A junction of the virus genome. Genotypes I, II, and III contain strains associated with human infections, with the majority of human strains grouped within genotypes I and III. Genotypes I-III have been further divided into sub-genotypes A and B for each genotype. Most non-human primate strains are grouped within genotypes IV, V, and VI. Despite the identification of multiple genotypes/strains, this is the only known serotype for HAV. Humans and several species of non-human primates are the only known natural hosts for HAV.

HAV is classified with the enterovirus group of the Picornaviridae family, genus *Hepatovirus*, and is comprised of single positive-stranded RNA genome of approximately 7.5 kilobases. This RNA molecule is protected from the environment by a protein capsid ("shell") comprised of multiple copies of

three or four proteins. HAV is a non-enveloped (i.e., no lipid-containing envelope), hydrophobic virus 22 to 30 nm in size, with icosahedral symmetry with 20 sides.

2. Disease

- **Mortality**: The overall death rate among people with hepatitis A (that is, liver involvement; the term "hepatitis A" is used to refer to the disease, not to the virus) is approximately 2.4%. Increased age (over 50 years old) slightly increases the death rate. Overall, hepatitis A accounts for < 0.001% of all foodborne-associated deaths. Although fulminant (severe, rapidly progressing) disease is rare, the mortality rate is much higher, at 70% to 80%, as noted in the Illness / complications section, below.

- **Infective Dose:** The infective dose of HAV is presumed to be low (10 to 100 viral particles), although the exact dose is unknown. The viral particles are excreted in the feces of ill people (symptomatic and asymptomatic) at high densities (10^6 to 10^8/gm) and have been demonstrated to be excreted at these levels for up to 36 days post-infection.

- **Onset:** In symptomatic patients, mean incubation phase is 30 days (range 15 to 50 days).

- **Illness / complications:** HAV infections can be asymptomatic or symptomatic. Infections usually are asymptomatic in children younger than age 6 and symptomatic in older children and adults.

  When disease does occur, it is usually mild and recovery is complete within 1 to 2 weeks, although it may last up to several months, in which case it is also generally self-limiting. HAV infection is not considered to be chronic; however, a prolonged or relapsing disease lasting up to 6 months in 10-15% of patients has been reported. Patients feel chronically tired during convalescence, and their inability to work can cause financial loss.

  An atypical, and rare, clinical outcome of acute HAV infection is fulminant hepatitis or fulminant hepatic disease, which occurs in less than 1% to 1.5% of cases. This more severe outcome of acute HAV infection and illness involves massive hepatic necrosis, with acute liver failure, and has a high case-fatality rate (70% to 80%).

  The reasons for progression to acute, severe, or fulminant hepatitis remain unclear; however, it is known that patients with an underlying chronic liver disease are at particularly high risk for fulminant disease or liver failure. Factors that may play a role in severe hepatic disease progression include the nature of the host response (e.g., genetic, immunologic, or physiologic), the viral pathogen (e.g., strain virulence), and/or viral dosage (e.g., viral inoculums, patient viral load, or levels of viral replication).

  A hepatitis A vaccine is available.

- **Symptoms:** Symptoms associated with HAV infection include fever, anorexia, nausea, vomiting, diarrhea, myalgia, hepatitis, and, often, jaundice. Jaundice generally occurs 5 to 7 days after onset of gastrointestinal symptoms; however, in 15% of reported jaundice cases, the jaundice was not preceded by gastrointestinal symptoms.

- **Duration:** Typically 1 to 2 weeks, although prolonged or relapsing cases may continue for up to 6 months in a minority of patients.

- **Route of entry:** HAV may cause infection through various routes. The route of entry for the foodborne infection is oral.

- **Pathway:** The exact mechanism of HAV pathogenesis is not fully understood. The route of entry for foodborne HAV typically is the gastrointestinal tract. From the intestinal tract, the virus is transported to the liver via the blood, where hepatocytes generally are thought to be the site of viral replication. The virus is thought to be excreted by the hepatocytes and transported to the intestinal tract via bile. However, some studies suggest that initial replication may occur in crypt cells of the small intestine.

3. Frequency

An estimated 1,566 cases of hepatitis A from consumption of contaminated food occur annually in the United States. This constitutes a small portion (1% to 1.5%) of the total number of patients infected with HAV. Overall, hepatitis A accounts for < 0.001% of all foodborne-associated hospitalizations in the U.S. Hepatitis A from any cause (i.e., not just the foodborne illness) has a worldwide distribution occurring in both epidemic and sporadic fashion. In the U.S., from 1980 through 2001, an average of 25,000 cases of hepatitis A was reported to the Centers for Disease Control and Prevention (CDC) annually. However, correcting for under-reporting and asymptomatic infections, CDC estimates that an average of 263,000 HAV infections, from all causes, occurred annually in the U.S. during this period.

Until 1995, the overall incidence of HAV infection in the U.S. was cyclic, with nationwide increases occurring every 10 to 15 years (Figure 1). Since 1995, the estimated overall number of reported HAV infections in the U.S. has been declining. This significant decrease (with the most significant decrease occurring in children) appears to coincide with the vaccination program, for children and adolescents 2 to 12 years old, that began in the U.S. in 1996.

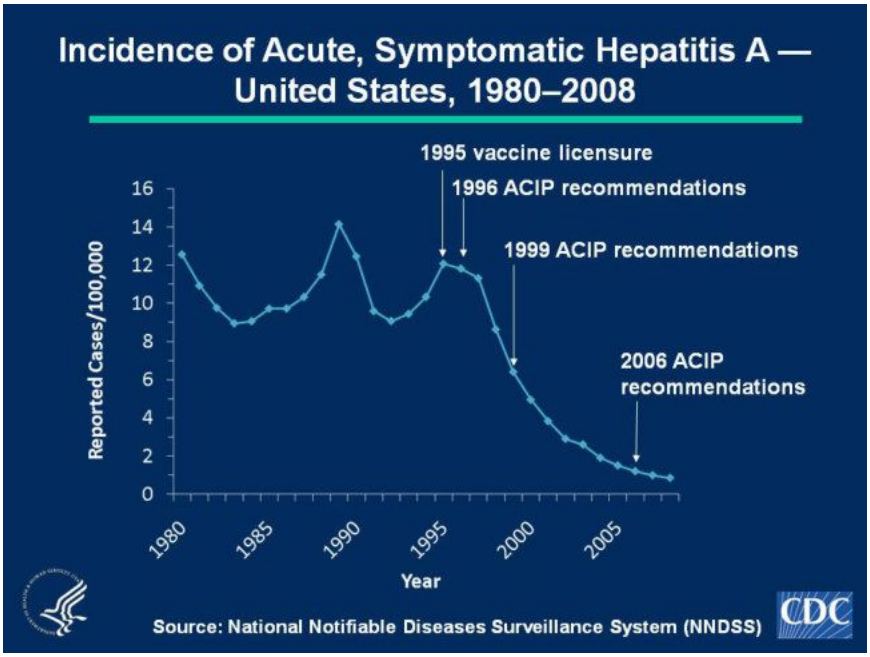


Figure 1. Centers for Disease Control and Prevention. Accessed May 2011.

4. Sources

HAV is excreted in feces of infected people and can produce clinical disease when susceptible people consume contaminated water or foods. Cold cuts and sandwiches, fruits and fruit juices, milk and milk products, vegetables, salads, shellfish, and iced drinks are commonly implicated in outbreaks. Water, shellfish, and salads are the most frequent sources. Contamination of foods by infected workers in food-processing plants and restaurants also is common.

In the U.S., the estimated transmission rate of this virus by person-to person contact was 22%. Of that, 8% was associated with day-care settings, 5% with international travel, 5% with illegal injectable drug use, and 4% with consumption of common-source contaminated food or water. The transmission routes for 65% of cases are unknown. Low income, low education level, crowding, and lack of access to safe drinking water and sanitation facilities are associated with increased rates of HAV infection.

5. Diagnosis

Clinical diagnosis of an HAV infection can be achieved by performing the appropriate analytical tests on serum or stool specimens. HAV diagnosis is generally performed by immunoglobulin (Ig) anti-hepatitis A antibody tests, IgM or IgG, in which an increase in virus-specific serum antibody titers is indicative of a recent HAV infection. One notable limitation for these antibody-based tests is that they cannot readily distinguish a recent HAV infection from increased antibody titer due to immunization, which can lead to elevated IgG and/or IgM being elicited against HAV. In addition to antibody testing, which also includes the use of immunoelectron microscopy, the use of molecular tests premised on reverse transcription polymerase chain reaction (RT-PCR) can also be utilized. Commercial kits are available to assist in HAV diagnosis.

6. Target Populations

All people are considered susceptible to HAV infection. Immunity can be developed by exposure and/or immunization that elicit an immune response that confers long-term immunity. In the U.S., the percentage of adults with immunity increases with age (10% for those 18 to 19 years of age to 65% for those over 50 years old). The increased number of susceptible people allows common-source epidemics to evolve rapidly.

7. Food Analysis

Methods have been developed to detect HAV in the food commodities most often implicated in HAV-associated illnesses; most notably, produce and shellfish. The manner in which the food is analyzed is dependent on the presumed location of contamination. For example, produce methods generally use a method to wash the viruses from the surface, whereas shellfish methods extract the virus from the digestive tract. Following extraction, the viruses are concentrated to suitable levels, so that detection via RT-PCR can be performed. These methods currently used by specialized regulatory laboratories to analyze suspected food for HAV are undergoing rigorous validation to verify that they are suitable for routine analysis.

8. Examples of Outbreaks

Hepatitis A is endemic throughout much of the world. Major national epidemics occurred in 1954, 1961, and 1971. Foods continue to be implicated in HAV outbreaks, which continue to occur in the U.S. following consumption of contaminated produce and shellfish. The most notable recent HAV outbreaks, in the U.S., that were associated with foods include:

- 1987 - Louisville, Kentucky- lettuce (imported)
- 1998 - Ohio- green onions (Mexico/California)
- 2000 - Kentucky and Florida- green onions (from Mexico) or tomatoes (California)
- 2003 - Tennessee, North Carolina, Georgia, Pennsylvania – green onions (Mexico)
- 2005 - Tennessee, Alabama – oysters (Louisiana)

Case Example: In August 2005, at least 10 clusters of hepatitis A illness, totaling 39 people, occurred in four states among restaurant patrons who ate oysters. Epidemiologic data indicated that oysters were the source of the outbreak. Trace-back information showed that the implicated oysters were harvested from a specific Gulf Coast shellfish-growing area. A voluntary recall of oysters was initiated in September. HAV was detected in multiple 25-gm portions in one of two recalled samples, indicating that as many as 1 of every 15 oysters from this source was contaminated (Shieh, 2007).

Other examples include:

CDC Morbidity and Mortality Weekly Report: Hepatitis A Virus
Provides a list of CDC Morbidity and Mortality Weekly Reports relating to this organism.

NIH/PubMed: Hepatitis A Virus
Provides a list of research abstracts contained in the National Library of Medicine's MEDLINE database for this organism.

Agricola: Hepatitis A Virus
Provides a list of research abstracts contained in the National Agricultural Library database.

9. Other Resources

- Shieh YC, Khudyakov YE, Xia G, Ganova-Raeva LM, Khambaty FM, Wood JW, Veazey JE, Motes ML, Glatzer MB, Bialek SR, and Fiore AE. 2007. *Molecular confirmation of oysters as the vector for hepatitis A in a 2005 multistate outbreak.* J. Food Prot. 70:145-150.

- HAV Definition and MeSH headings from the National Library of Medicine

Bad Bug Book

Foodborne Pathogenic Microorganisms and Natural Toxins

Hepatitis E virus

1. Organism

While hepatitis E Virus (HEV) is considered to be labile when not in the acidic conditions found in the gastrointestinal tract or in fecal material, studies have demonstrated that it can withstand thermal inactivation at temperatures near those expected to be found within a rare-cooked steak (approximately 57°C). HEV is more labile than is the hepatitis A virus (HAV), and the levels of viable virus decrease rapidly at higher temperatures. Repeated freezing and thawing can gradually decrease the levels of any infectious virus, and HEV is no different, but also, perhaps, not worse, when compared with other enteric viruses. Moreover, like other enteric viruses, HEV does not have a lipid envelope, which contributes to its ability to somewhat withstand exposure to alcohols and detergents. HEV does seem especially susceptible to high salt concentration.

As with HAV, HEV growth in cell culture is poor. However, HEV has a much more extensive host range, including primates, pigs, rats, cattle, chicken, and sheep. Existing microbiological and epidemiologic data suggest a potential role of swine in the human transmission of HEV: global existence of anti-HEV seropositive swine, genetic relatedness of swine and human isolates, interspecies transmission of swine and human strains,

For Consumers: A Snapshot

Hepatitis E is caused by a virus. It's not very common in the U.S., but is common in some areas of the world with poor sanitation. Most people who get it are mildly sick for a couple of weeks, and the illness goes away by itself – but pregnant women tend to get much sicker from hepatitis E and are much more likely to die from it. People with weak immune systems also may get sicker than others and are more likely to get the illness for much longer or permanently. Examples include people with HIV/AIDS and people who are on certain medications meant to lower the actions of the immune system (like some drugs for rheumatoid arthritis or cancer, or drugs given after an organ transplant). Like other forms of hepatitis, this one causes inflammation of the liver. Symptoms of hepatitis E may include a tired, sick feeling; low appetite; pain in the stomach and the joints; enlarged liver; yellow skin and eyes; and fever. In pregnant women, especially, the disease can cause very serious liver damage and can destroy the liver. Although contaminated food could pass this virus to people, the main way it gets into people is from the hands into the mouth. For example, when infected people have a bowel movement and don't wash their hands well afterwards, or when people clean an infected person who has had a bowel movement and don't wash their hands well, they can spread the virus to anything they touch, and other people can pick it up when they touch that same surface later. Water contaminated with feces (sewage) from humans or swine (pigs) is a common way that the virus is passed to people; for example, if people drink the water, or if they eat fruits or vegetables that were irrigated or washed with it. There is no vaccine for hepatitis E, yet (although there are vaccines for other forms of hepatitis), but you can help protect yourself by following basic food-safety tips. Examples that are especially important for preventing hepatitis E include washing hands well after having a bowel movement or cleaning someone else who has had one; using only bottled water if you travel to countries with poor sanitation; washing raw fruits and vegetables under running water; and thoroughly cooking meat that came from wild game (such as deer or boars) and pigs, since the virus has been found in these animals.

recovery of HEV from pork products implicated in disease outbreaks, and high seroprevalence levels among swine caretakers.

Hepatitis E virus has a particle diameter of 32-34 nm, a buoyant density of 1.29 g/ml in KTar/Gly gradient, and, under some circumstances, is very labile. It has a positive-sense, single-stranded polyadenylated RNA genome of approximately 7.2 kb, with three open reading frames (ORFs). ORFs 1-3 encode the non-structural proteins (e.g., RNA polymerase and helicase), the capsid protein, and a small immunogenic protein that may play a role in virus particle assembly, respectively. ORF 1 is near the 5′ end of the viral genome, but does not overlap with ORF 2. Instead ORF 3 begins at the very end of ORF 1 and overlaps with ORF 2, which is toward the 3′ end of the genome.

While the icosahedral shape of the capsid, size of the virus particle, lack of outer lipid envelope, and size of the viral genome suggests a resemblance to other fecally transmitted viruses, such as hepatitis A (HAV) and norovirus, hepatitis E has some distinguishing physicochemical and genetic properties. Based on such properties, the virus recently was assigned its own genus (Hepevirus) and family (Hepeviridae). At least five genotypes exist [human, swine (1-4) and avian (5)], with only a single serotype recognized. Genotype 3 can be found in swine worldwide and is the strain involved in autochthonous transmission resulting in mild, if any, symptoms and disease in humans.

2. Disease

HEV is a known cause of epidemic and intermittent (sporadic) cases of enterically-transmitted acute hepatitis. The disease caused by HEV is called hepatitis E, or enterically transmitted non-A non-B hepatitis (ET-NANBH). Other names include fecal-oral non-A non-B hepatitis, and A-like non-A non-B hepatitis. Hepatitis E was acknowledged as a distinct disease only as recently as 1980.

Since there is no specific treatment for hepatitis E, other than treatment of symptoms, prevention is the best course of action.

Note: This disease should not be confused with hepatitis C, also called parenterally transmitted non-A non-B hepatitis (PT-NANBH), or B-like non-A non-B hepatitis, which is a common cause of hepatitis in the United States.

- **Mortality:** The fatality rate is 0.5 to 4%, except in pregnant women, in whom case-fatality rates can reach 27%. Death usually occurs in those with previous liver disease.

- **Infective dose:** The infective dose is not known.

- **Onset:** Incubation period following exposure can range from 3 to 8 weeks, with a mean of 5.7 weeks.

- **Illness / complications:** Hepatitis caused by HEV is clinically indistinguishable from hepatitis A disease. The disease usually is mild and self-resolves in 2 weeks, with no sequelae. However, chronic hepatitis has been reported in organ transplant recipients and in patients with active HIV infections. Epidemiologic studies have established an association between HEV-infected pregnant women and incidences of fatal fulminant hepatic failure.

- **Symptoms:** Symptoms are most often seen in patients between the ages of 15 to 40, but, in younger children, the absence of symptoms, including jaundice, is common and results in infections not being recognized and documented. Symptoms include jaundice, malaise, anorexia, abdominal pain, arthralgia, hepatomegaly, vomiting, and fever.

- **Duration:** Extended viremia and fecal shedding are not typical. The disease usually is mild and self-resolves in 2 weeks, with no sequelae. Virus excretion has been noted as long as 2 weeks after jaundice appears, but peaks during the incubation period, as does viremia. Notably, HEV is shed in lower titers than is HAV.

- **Route of entry:** HEV is transmitted by the fecal-oral route. Person-to-person spread is not common. Pig-organ and human liver transplantations and blood transfusions may also be involved in HEV transmission.

- **Pathway:** The pathogenic pathway for HEV is not completely understood. After the consumption of contaminated food or water, the virus reaches the liver from the intestinal tract, but the exact route and mechanism are not clear. From studies conducted in infected non-human primates and swine, we know that HEV primarily replicates in gall-bladder and liver cells. Replication also has been established in extrahepatic sites, such as the small intestine, lymph nodes, colon, and salivary glands. However, evidence of viral replication has not been documented in the spleen, tonsil, or kidney. Some of the highest virus load has been noted in bile samples. The injury to the liver sometimes noted following infection could be related to triggered immunological responses and (possibly), additionally, to morphological changes (cytopathic effects) caused by the virus invading liver cells.

3. Frequency

Epidemic hepatitis E is primarily a disease of concern in developing countries, due to inadequate public sanitation infrastructure (inadequate treatment of drinking water and sewage). Notably, within developing countries, the majority of sporadic cases of viral hepatitis can be attributed to HEV, rather than to the other major hepatotropic viruses (hepatitis A, B, or C). Major waterborne epidemics have occurred in Asia and North and East Africa. Locally acquired (autochthonous) cases of hepatitis E in industrialized countries, including the U.S. and Europe, are increasing. Seroprevalence studies in the U.S. and Europe report a 1% to 25% prevalence of HEV antibodies in healthy individuals. Reports suggest that cases of HEV disease in industrialized countries are autochthonous, largely overlooked, segregate into genotype III, and lack a precise source of infection.

[Also see Diagnosis section, below, regarding likely under-diagnosis among immunocompromised people.]

4. Sources

Waterborne and foodborne transmission have been documented. For example, zoonotic spread involving group consumption of undercooked wild boar meat has been recognized in Japan, and viable HEV has been recovered from commercially sold pork livers in the U.S. Infectious HEV also has been isolated from swine feces and stored waste material. Food safety concerns arise when human and swine agricultural waste is used for irrigation of produce, such as tomatoes and strawberries, likely to be eaten raw and potentially without washing, or when such waste contaminates waters where shellfish are harvested. Evidence exists that implicates shellfish as a

foodborne source of infection for two of the eight cases of HE identified in the UK, in 2005. In Europe and the U.S., HEV has been recovered from municipal sewage. Figatellu, a pig liver sausage commonly eaten raw in France, also has been recently implicated in hepatitis-E-related disease. Because of the increasing trend in the U.S. to both hunt and eat wild boar meat and evidence suggesting that these animals can harbor HEV, the proper handling of the carcass and thorough cooking of any meat should be considered.

5. Diagnosis

Diagnosis of HEV disease is based on the epidemiologic characteristics of an outbreak and by exclusion of hepatitis A and B viruses by serological tests. Confirmation requires identification of the 27-34 nm virus-like particles, by immune electron microscopy, in feces of acutely ill patients or by molecular detection of genomic RNA in serum or feces. Because of the dangers of rapidly progressing, severe disease in pregnant women, hospitalization should be considered. Since HEV infection can often cause mild, if any, symptoms in immunocompetent individuals, this disease is largely under-diagnosed in developed countries.

6. Target Populations

The disease is most often seen in young to middle-age adults (15 to 40 years old). Pregnant women appear to be exceptionally susceptible to severe disease, and excessive mortality has been reported in this group. Immunocompromised people are at risk of chronic HEV disease. High anti-HEV seroprevalence rates have been seen in those occupationally in close contact with swine.

7. Food Analysis

No method is currently available for routine analysis of foods.

8. Examples of Outbreaks

For information about recent outbreaks, see the CDC's Morbidity and Mortality Weekly Reports.

9. Other Resources

- Loci index for genome Hepatitis E
- CDC/MMWR: Hepatitis E Virus – provides a list of Morbidity and Mortality Weekly Reports at CDC relating to this organism or toxin.
- NIH/PubMed: Hepatitis E Virus – provides a list of research abstracts contained in the National Library of Medicine's MEDLINE database.
- Agricola: Hepatitis E Virus – provides a list of research abstracts contained in the National Agricultural Library database for this organism.

Bad Bug Book

Foodborne Pathogenic Microorganisms and Natural Toxins

Rotavirus

1. Organism

Human rotaviruses (HRV) are quite stable in the environment and have been found in estuary samples at levels as high as 1 to 5 infectious particles/gallon. Sanitary measures adequate for bacteria and parasites seem to be ineffective for endemic control of rotavirus, as similar incidence of rotavirus infection is observed in countries with both high and low health standards. Rotaviruses are stable in a wide pH range, with the infectivity being unaltered at pH of 3 to 11, but rapidly inactivated at pH of 2.5 and below or at 11.5 and above. They are stable at low temperatures of -20°C and 4°C, with minimal loss of titer after 32 days, and are stable during 6 freeze / thaw cycles. Rotaviruses are stable for up to 4 days at 37°C and rapidly inactivated at 56°C. Rotaviruses are inactivated by UV light and by disinfectants, including chlorine, H_20_2, and ethanol.

These viruses belong to a genus of double-stranded RNA viruses in the Reoviridae family. They have a genome consisting of 11 double-stranded RNA segments surrounded by three protein layers. The outer protein layer is composed of VP4 and VP7; the middle layer is composed of VP6; and an inner layer is composed of VP2. Six serological groups have been identified, three of which (groups A, B, and C) infect humans.

2. Disease

Rotavirus is among the leading causes of diarrhea and dehydration in children, worldwide. In the United States, the occurrence of rotavirus has dropped considerably since introduction of a vaccine in 2006.

For Consumers: A Snapshot

Anyone, of any age, can become sick from rotavirus, but it's especially a problem for infants and children. It's one of the main causes of diarrhea and dehydration (losing too much body fluid) in this age group. Although the illness usually is mild, and most people get better, it causes half a million deaths in children younger than 5 years old, worldwide, each year. Since 2006, a rotavirus vaccine has been given to children in the U.S., and rotavirus illness in this country has gone down. Although contaminated food can pass this virus to people, the main way it gets into people is from the hands into the mouth. For example, when an infected person goes to the bathroom and doesn't use good handwashing afterwards, anything he or she touches – such as a doorknob – can become contaminated with the virus, and another person can pick it up on his or her hands; then it can get into the mouth through food or touching. When food causes this illness, it's likely to be food that was handled by an infected person and then wasn't cooked, such as salads and raw fruits and vegetables. Watery diarrhea starts in about 2 days, and other symptoms may include vomiting and fever higher than 101° F. Most people get better in 3 days to a week. But the illness may be much more serious in some people, especially very young children, premature babies, elderly people, and people with weak immune systems or who are on certain medicines, such as some drugs used for rheumatoid arthritis. It's especially important for these people to go to a health professional, even though antibiotics don't work against viruses. Losing so much fluid through diarrhea can throw off the body's balance in serious ways that can lead to death. A health professional can return the body to the right balance with treatments of fluids and certain minerals. To help prevent illness from rotavirus, get your children vaccinated, wash your hands after using the bathroom or handling diapers, and follow other basic food-safety tips.

- **Mortality**: Childhood mortality caused by rotavirus is relatively low in the U.S., with an estimated 20 to 60 deaths per year, but reaches approximately 0.5 million deaths per year, worldwide. A recent CDC report estimates that, in the U.S., there are zero deaths annually from domestically acquired rotavirus.

- **Infective dose:** The infective dose is presumed to be 10 to 100 infectious viral particles.

- **Onset**: The incubation period for rotavirus is estimated to be less than 48 hours.

- **Illness / complications:** Rotaviruses cause acute gastroenteritis, usually with complete recovery. Infantile diarrhea, winter diarrhea, acute nonbacterial infectious gastroenteritis, and acute viral gastroenteritis are names applied to the infection caused by the most common and widespread group A rotavirus. Temporary lactose intolerance may occur.

  Rotavirus is shed in large numbers (10^{12} infectious particles/ml of feces) before, and for several days after, symptoms resolve. Infectious doses can be readily acquired through contaminated hands, objects, or utensils. Asymptomatic rotavirus excretion has been well documented and may play a role in perpetuating endemic disease.

- **Symptoms**: Rotavirus gastroenteritis has symptoms ranging from self-limiting, mild, watery diarrhea, with complete recovery, to severe disease characterized by vomiting, watery diarrhea, and fever, which can lead to dehydration, hypovolemic shock, and, in severe cases, death. Symptoms often start with a fever (greater than 101ºF) and vomiting, followed by diarrhea. Severe diarrhea without fluid and electrolyte replacement may result in severe dehydration and death. Association with other enteric pathogens may also play a role in the severity of the disease.

- **Duration**: Diarrhea generally lasts 3 to 7 days.

- **Route of entry**: Rotaviruses are transmitted via the fecal-oral route. Infected food handlers may contaminate foods that require handling without further cooking. However, person-to-person spread through contaminated hands is probably the most important means by which rotaviruses are transmitted in close communities, such as pediatric and geriatric wards, day-care centers, and family homes.

- **Pathway**: Rotavirus infects the mature absorptive enterocytes in the ileum and causes diarrhea by virus-associated cell death and release of a non-structural protein, which may trigger an intracellular calcium-dependent signaling pathway. Rotavirus may activate secretomotor neurons of the enteric nervous system that stimulate secretion of fluids and solutes.

3. Frequency

Group A rotavirus is endemic worldwide and is the leading cause of severe diarrhea among infants and young children, accounting for about half of the cases requiring hospitalization. More than 3 million cases of rotavirus gastroenteritis occur annually in the U.S.; of these, 15,433 cases are foodborne, according to a recent estimate by the Centers for Disease Control and Prevention (CDC). In temperate areas, it occurs primarily in the winter, but in the tropics, it

occurs throughout the year. The number of cases attributable to food contamination is unknown, but this route of transmission is thought to be rare.

After the introduction of a vaccine for rotavirus in 2006, the CDC found that rotavirus activity during 2007 and 2008 was substantially lower than that reported during 2000-2006.

Group B rotavirus, also called adult diarrhea rotavirus or ADRV, has caused major epidemics of severe diarrhea affecting thousands of persons, of all ages, in China.

Group C rotavirus has been associated with rare and sporadic cases of diarrhea in children in many countries. However, the first outbreaks were reported from Japan and England.

4. Sources

As noted, person-to-person fecal-oral spread is the most important means of transmission, but foods such as salads, fruits, and hors d'oevres that do not require further cooking and are handled by an infected food worker also may transmit rotaviruses.

5. Diagnosis

Rotavirus cannot be diagnosed by clinical symptoms alone. Laboratory testing of stool samples is required for a diagnosis of rotavirus, although it is generally not done. The most common laboratory tests that are available are enzyme immunoassays (EIA) and latex agglutinations (LA). EIA is the test most widely used to screen clinical specimens, and several commercial kits are available for group A rotavirus. Other assays include electron microscopy (EM) and culture and molecular techniques, including reverse transcriptase polymerase chain reaction (RT-PCR).

6. Target Populations

Humans of all ages are susceptible to rotavirus infection. Children 3 months to 2 years old, premature infants, the elderly, and the immunocompromised are particularly prone to more severe symptoms caused by infection with group A rotavirus.

7. Food Analysis

To date, the virus has not been isolated from any food associated with an outbreak, and no satisfactory method is available for routine analysis of food. However, it should be possible to apply procedures that have been used to detect the virus in water and in clinical specimens, such as RT-PCR, to food analysis.

8. Examples of Outbreaks

The CDC's MMWR describes an outbreak that appears to have been foodborne, initially, then spread through person-to-person contact.

9. Other Resources

- NCBI taxonomy browser
- CDC information about rotavirus

Other Viral Agents

For Consumers: A Snapshot

Of the viruses that can cause illness through contaminated food, norovirus, hepatitis, and rotavirus cause the largest number of known cases. They're covered in separate chapters of this book. This chapter is about other viruses that also cause foodborne illness, but not nearly as often. In general, the illnesses they cause start within 10 to 70 hours after a person eats or drinks contaminated food or fluid, are mild, last anywhere from 2 to 9 days, and go away by themselves. Some common symptoms are nausea; vomiting; diarrhea; a sick, uncomfortable feeling; abdominal pain; headache; and fever. Following basic food-safety tips can help protect you from getting these viruses. Since they can also be spread from person to person (for example, when infected people have a bowel movement and don't wash their hands well, so that anything they touch spreads the virus to other people and objects), good handwashing is especially important.

1. Organisms

Although rotavirus and norovirus are the leading causes of viral gastroenteritis, a number of other viruses have been implicated in outbreaks, including astroviruses, Sapovirus, enteric adenoviruses, parvovirus, and Aichi virus.

- **Astroviruses** are classified in the family Astroviridae. Human astroviruses (HAstVs) contain a single positive strand of RNA of about 7.5 kb surrounded by a protein capsid of 28-30 nm diameter. A five- or six-pointed star shape can be observed on the particles under the electron microscope. Mature virions contain two major coat proteins of about 33 kDa each. There are eight serotypes, HAstVs-1 to HAstVs-8, with HAstVs-1 being most frequently associated with viral gastroenteritis.

- **Sapoviruses** (SaV) are classified in the family Caliciviridae. They contain a single strand of RNA, about 7.5kb, surrounded by a protein capsid of 41-46 nm diameter. Mature virions have cup-shaped indentations, which give them a "Star of David" appearance in the electron microscope. The viruses contain a single major coat protein of 60 kDa. Five serotypes (GI-GV) have been identified, with GI, GII, GIV, and GV causing gastroenteritis in humans.

- **Enteric adenoviruses (HuAd)** are classified in the family Adenoviridae. These viruses contain a double-stranded DNA, about 35 kb, surrounded by a distinctive protein capsid non-enveloped icosahedral shell of about 90-100 nm in diameter. Of the 51 serotypes of human Adenoviruses, the serotypes most prevalent in gastroenteritis are 40 and 41, but 12, 18, and 31 also cause gastroenteritis.

- **Parvoviruses**, including Human Bocavirus (HBoV), are members of the Bocavirus genus of the Parvoviridae, belong to the family Parvoviridae, the only group of animal viruses to contain linear single-stranded DNA. The DNA genome is surrounded by a protein capsid of about 22 nm in diameter.

- **Aichi virus (AiV)** is classified in the family Picornaviridae family as a member of the Kobuvirus genus. They contain a single strand of RNA, of about 8.3 kb. Aichi virus isolates have been divided into groups 1 (genotype A) and 2 (genotype B).

2. Disease

Common names of the illness caused by these viruses are acute gastroenteritis (AGE), acute nonbacterial infectious gastroenteritis and viral gastroenteritis.

- **Mortality**: Unknown.
- **Infective dose:** The infective dose of these viruses generally is not known, but is presumed to be low.
- **Onset**: Usually 10 to 70 hours after contaminated food or water is consumed.
- **Illness / complications:** Viral gastroenteritis is usually a mild, self-limiting illness. The clinical features are milder, but otherwise indistinguishable from, rotavirus gastroenteritis. Co-infections with other enteric agents may result in more severe illness that lasts longer.
- **Symptoms**: May include nausea, vomiting, diarrhea, malaise, abdominal pain, headache, and fever.
- **Duration**: Generally 2 to 9 days.
- **Route of entry**: Ingestion of contaminated food (or fecal-oral route, via person-to-person contact).
- **Pathway**: The infectious pathway for these viral agents is intestinal mucosal tissues and adenovirus may involve the respiratory track.

3. Frequency

Astroviruses cause sporadic gastroenteritis in children under 4 years of age and account for about 4% of the cases hospitalized for diarrhea. Most American and British children over 10 years of age have been found to have antibodies to the virus.

Sapoviruses cause a sporadic gastroenteritis similar to norovirus in populations ranging from children to the elderly. The infections are more frequent in children under age 5 than in adults.

Enteric adenoviruses cause 5% to 20% of the gastroenteritis in young children and are the second most common cause of gastroenteritis in this age group. By 4 years of age, 85% of all children have developed immunity to the disease.

Bocaviruses have been implicated in sporadic cases of gastroenteritis in children and adults, with 0.8 to 9.1% of stools screening positive for bocaviruses.

Aichi virus has been associated with sporadic outbreaks in children and adults in Asian countries and Brazil.

4. Sources

Viral gastroenteritis is transmitted by the fecal-oral route via person-to-person contact or ingestion of contaminated foods and water. Food handlers may contaminate foods that are not further cooked before consumption. Enteric adenovirus may also be transmitted by the respiratory route. Shellfish have been implicated in illness caused by many of these viruses.

5. Diagnosis

Clinical diagnosis of these viruses can be achieved by performing the appropriate molecular methods on stool or serum. Identification of the virus present in early, acute stool samples is made by immune electron microscopy and various enzyme immunoassays. Confirmation often requires demonstration of seroconversion to the agent by serological tests on acute and convalescent serum pairs. Commercial kits are available for astroviruses.

6. Target populations

The target populations for these viruses are young children and the elderly, with sporadic outbreaks occurring among all populations. Infection with these viruses is widespread and seems to result in development of immunity.

7. Food Analysis

Although foods are not routinely analyzed for these viruses, molecular techniques, such as RT-PCR, have been developed to identify all of the above viruses. Detection methods, coupled with the extraction methods developed for norovirus and other enteric foodborne viruses, can be used or adapted to detect the viruses in food.

8. Examples of Outbreaks

Le Guyader FS, Le Saux JC, Ambert-Balay K, Krol J, Serais O, Parnaudeau S, Giraudon H, Delmas G, Pommepuy M, Pothier P, Atmar RL. 2008. Aichi Virus, Norovirus, Astrovirus, Enterovirus, and Rotavirus Involved in Clinical Cases from a French Oyster-Related Gastroenteritis Outbreak. J. Clin Micro, 46(12): 4011-4017.

9. Resources

- NCBI taxonomy browser
- CDC information about viruses

Other Pathogenic Agents

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Prions and Transmissible Spongiform Encephalopathies

1. Organism

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases. There are examples of these diseases in both humans and animals. The spongiform portion of their name is derived from the fact that microscopic analysis of the affected brain tissue shows the presence of numerous holes, which gives the brain a sponge-like appearance. The disease-causing entity that elicits all TSEs is neither a cellular organism (i.e., a bacterium or parasite) nor a virus. Rather, it is the prion protein, a normal mammalian cell protein, that causes these diseases.

Under normal physiologic conditions, the prion protein is found on the surface of a wide variety of cells within the body, most notably in nervous tissue, such as nerve cells and brain tissue. While our understanding of the precise function of this protein is still evolving (and somewhat controversial), current evidence suggests that prions have a role in long-term memory and/or maintaining normal nerve-cell physiology. Prion diseases are initiated when normal cellular prions come in contact with a disease-causing prion. The disease-causing prion is a misfolded form of the normal prion. Once it is misfolded, it can induce other, normally folded prion proteins to become misfolded. This folding / misfolding process is responsible for the amplification of disease.

For Consumers: A Snapshot

Prions (pronounced "PREE – ons") aren't living things, but may cause a certain type of rare, deadly disease if infected cattle are eaten. Prion disease in cattle isn't common (there have been only three known cattle cases in the U.S.) and affects the brain, some nerves, the spinal cord, eyes, tonsils, and bowel. Since 1996, when it first appeared in humans, only 217 people in the world are known to have gotten the disease, whose medical name is "variant Creutzfeldt-Jakob disease," shortened to vCJD. It's thought that the meat these people ate was contaminated because the cattle had been given feed that contained parts of other, dead cattle (as a protein source) that were contaminated with disease-causing prions. Since that kind of cattle feed has been banned, the number of new cases has dropped even lower. In both humans and cattle, disease-causing prions are a protein that has taken on the wrong shape. Normally, the correctly-shaped prion protein helps the brain and nerves work properly, but when it takes on the wrong shape it can result in vCJD in humans. Once meat from diseased cattle is eaten and diseased prions enter a person's system, they turn the normal prions into disease-causing prions, and the brain and nerves no longer work properly, leading to death. It's thought that symptoms don't appear until about 10 years after the infectious meat is eaten. The illness may begin with depression or other psychiatric problems and develop into neurologic symptoms, such as unpleasant feelings in the face, arms, and legs, and trouble understanding, remembering, talking, and walking, which becomes extreme. Although this disease made headlines when it appeared in the mid-1990s, it's important to remember that, of the entire population of the world, only 217 cases of vCJD have been reported, and added safety regulations for feeding cattle appear to be working to prevent the disease. There have been only three cases of vCJD in the U.S. All three were linked *not* to the three U.S. cattle that had been found to carry disease prions, but instead to contaminated meat the three people had eaten while overseas.

There are several naturally occurring human TSEs: Kuru, Fatal Familial Insomnia, Gerstmann-Straussler-Scheinker Syndrome, and Creutzfeldt-Jakob Disease (CJD). Kuru and CJD are the only human-specific TSEs that can be transmitted between people (although not through normal person-to-person contact, in either case, as described below). Kuru was spread only when the brains of individuals infected with this disease were eaten as part of ritual acts of mortuary cannibalism. Kuru and its unusual route of transmission were confined to the South Fore tribe in New Guinea; it is no longer transmitted, as the tribe no longer practices this portion of their death ritual. There are three different types of classic CJD; spontaneous, familial, and iatrogenic. Only iatrogenic, or acquired, CJD is transmissible. This form of CJD is transmitted through unintended exposure to infected tissue during medical events (for example, from *dura mater* grafts or from prion-contaminated human growth hormone). Spontaneous CJD accounts for approximately 85% of all CJD cases and occurs in people with no obvious risk factors. Familial, or hereditary, CJD is a disease passed from parent(s) to offspring and comprises approximately 10% of all CJD cases.

Only variant Creutzfeldt-Jakob Disease (vCJD) is transmitted through food. Variant Creutzfeldt-Jakob Disease and the cattle disease bovine spongiform encephalopathy (BSE), also known as "mad cow" disease, appear to be caused by the same agent. Other TSE diseases exist in animals, but there is no known transmission of these TSEs to humans. Included among these are chronic wasting disease (CWD) of deer and elk, and scrapie, the oldest known animal TSE, which occurs in sheep and goats.

No early, acute clinical indicators for TSEs have been described.

2. Disease

- **Mortality**: vCJD is always fatal. There is no known cure for this disease.
- **Infective dose:** The precise amount of disease-causing prions from BSE-infected tissue that is needed to cause disease in man is unknown. However, based on research studies in cattle, the amount needed to transmit disease is very small. In that research, as little as 1 ug (0.000000035 ounces) of brain tissue from a BSE-infected cow was needed to transmit the disease to an otherwise healthy cow. The normal “species barrier effect” toward infectivity will require a higher amount of infectious material to be consumed by people in order to transmit the disease to humans.
- **Onset**: It is believed that there is a lag time of approximately 10 years between exposure to the BSE-causing agent and development of clinical signs of vCJD. The age of onset for vCJD has ranged from as young as 16 years of age to 52 years of age. The median age is 28 years. (This is in contrast to classic CJD, in which the median age of onset is 68 years of age and is rarely found in people younger than 60 years of age.)
- **Illness / complications:** Variant Creutzfeldt-Jakob Disease is a progressively debilitating neurodegenerative disease.
- **Symptoms**: Cases of vCJD usually present with psychiatric problems, such as depression. As the disease progresses, neurologic signs appear, such as unpleasant sensations in the limbs and/or face. There are problems with walking and muscle coordination. Sometimes, late in the course of the disease, victims become forgetful, then experience severe problems with processing information and speaking. Patients are hospitalized and are increasingly unable to care for themselves, until death occurs.

- **Duration**: The length of disease in vCJD patients, from initial diagnosis to death, is on the order of months to years (up to 2 years; median 14 months), and the median age at death is 28 years of age. (In CJD patients, the length of time from initial diagnosis to death is weeks to months, median time 4.5 months, and the median age at time of death is 65 to 68 years.)
- **Route of entry**: The traditional route of entry into humans of the BSE-causing agent is oral, through consumption of meat or meat products derived from BSE-infected animals. Three individuals in Great Britain are believed to have contracted vCJD through blood transfusions from a single blood donor, who was subsequently diagnosed as vCJD-positive.

  The oral route is also how BSE is spread and disseminated in cattle. It was a standard practice to feed cattle rendered animal by-products, including rendered by-products from other cattle. It is believe that BSE was spread by feeding cattle the rendered by-products of BSE-infected cattle. This practice has now been banned and, along with enhanced surveillance of cattle populations for BSE, has led to the dramatic reduction in the number of cattle infected with BSE, and has indirectly been responsible for the corresponding reduction in the number of vCJD cases.

3. Frequency

A total of 217 people have contracted vCJD, worldwide.

More than 185,000 cattle worldwide have been infected with BSE. As of February 2011, there have been 22 cases of BSE in North America; 3 in the United States and 19 in Canada. One of the U.S. cattle and one of the Canadian cattle were born in Great Britain.

There is no known relationship between the number of BSE-infected cattle and the incidence of humans infected with vCJD.

4. Sources

Development of vCJD is believed to be the result of eating meat or meat products from cattle infected with BSE. The available scientific information strongly supports the supposition that the infectious agent that causes BSE in cattle is the same agent that causes vCJD in humans. (Also see “Food Analysis,” below.)

5. Diagnosis

Preliminary diagnoses of vCJD are based on patient history, clinical symptoms, electroencephalograms, and magnetic resonance imaging of the brain. The most definitive means for diagnosing any TSE is microscopic examination of brain tissue, which is a postmortem procedure.

6. Target Populations

All cases of vCJD, to date, have occurred in individuals of a single human genotype that is homozygous for the amino acid methionine at codon 129 of the prion protein. About 40% of the total human population belongs to this methionine-methionine homozygous state. The susceptibility of other genotypes is not yet known.

7. Food Analysis

The most effective means of preventing vCJD is to prevent the spread and dissemination of BSE in cattle. The prohibitions on feeding rendered cattle by-products to cattle have been very effective in helping reduce the number of new cases of BSE-infected cattle, worldwide.

The major concern for consumers is the potential contamination of meat products by the BSE causative agent or the inclusion of BSE-contaminated tissues in foods, including dietary supplements. There are no tests available to determine if food derived from cattle contain the BSE-causing agent. There are postmortem tests to determine if asymptomatic cattle are carrying the BSE disease-causing prions. High-risk tissues for BSE contamination include the cattle's skull, brain, trigeminal ganglia (nerves attached to the brain), eyes, tonsils, spinal cord, dorsal root ganglia (nerves attached to the spinal cord), and the distal ileum (part of the small intestine). The direct or indirect intake of high-risk tissues may have been the source of human illnesses in the United Kingdom and elsewhere. Bovine meat (if free of central nervous system tissue) and milk have, to date, shown no infectivity. Gelatin derived from the hides and bones of cattle appears to be very low risk, especially with adequate attention to the quality of source material and effectiveness of the gelatin-making process. Based on many studies, scientists have concluded that vCJD does not appear to be associated with consumption of specific foods.

8. Examples of Outbreaks

There has been only one outbreak of vCJD, which is still ongoing. The first case of vCJD was discovered in 1996, in Great Britain. Since then, a total of 217 patients worldwide have been diagnosed as having vCJD (through August 2010). A total of 170 patients have been diagnosed in Great Britain, with 25 cases in France, 5 in Spain, 4 in Ireland, 3 each in the U.S. and the Netherlands, 2 each in Portugal and Italy, and one each in Canada, Japan, and Saudi Arabia. Two of the three patients in the U.S. contracted vCJD while living in Great Britain, while the third patient most likely contracted this disease while living in Saudi Arabia. Three patients from Great Britain contracted vCJD following a blood transfusion from a single, asymptomatic vCJD blood donor. The peak number of new cases occurred in 2000, and the number of new cases has continued to decline in the subsequent years.

9. Resources

- Centers for Disease Control and Prevention information about vCJD – Provides information about vCJD, updated information on the ongoing number of clinical cases and other pertinent information about vCJD, with links to information about BSE.
- Centers for Disease Control and Prevention Morbidity and Mortality Weekly Reports: Prions and TSEs – Provides a list of MMWR relating to this organism.
- NIH/PubMed: Prions and TSEs – Provides a list of research abstracts contained in the National Library of Medicine's MEDLINE database for this organism.
- Agricola: Prions and TSEs – Provides a list of research abstracts contained in the National Agricultural Library database for this organism or toxin.
- Loci index for PrP of Homo sapiens
- GenBank Taxonomy database
- PrP Protein in humans
- PrP Protein in cattle

Natural Toxins

Ciguatoxin

1. Organism and Toxin

Dinoflagellates (marine algae) in the genus *Gambierdiscus* occur in certain tropical and subtropical areas of the world. These dinoflagellates elaborate ciguatoxins and/or precursors of the ciguatoxins called gambiertoxins. As these compounds are transmitted through the marine food web, they are concentrated and may be chemically altered. Ciguatoxins are not significantly affected by cooking or freezing.

2. Disease

Ciguatera fish poisoning is a human illness caused by consumption of subtropical and tropical marine finfish that have accumulated ciguatoxins through their diets.

- **Mortality:** There is a very low incidence of death, from respiratory and/or cardiovascular failure.
- **Toxic dose:** Not well established, and variable, since many different ciguatoxins, of different toxicities, may be present in a toxic fish. Probably less than 100 nanograms (100 billionths of a gram) is adequate to cause illness.
- **Onset:** Usually within 6 hours after consumption of toxic fish.
- **Illness / complications:** Ciguatera in humans usually involves a combination of gastrointestinal, neurological, and, occasionally, cardiovascular disorders. Symptoms defined within these general categories vary with the geographic origin of toxic fish, and to some extent, with the species of fish. There is no reliable, proven treatment for the poison.

For Consumers: A Snapshot

The large majority of fish are safe to eat and provide good nutrition. But if you plan to go fishing in tropical areas and plan to eat what you catch, be aware that some kinds of fish in those areas may contain a poison called "ciguatoxin." There's no way to tell if a fish contains ciguatoxin from the way it looks, tastes, or smells; the only way to tell is by testing in a professional laboratory. Cooking and freezing don't get rid of the poison. The illness usually starts within 6 hours after the fish is eaten. Symptoms and signs may include numbness and tingling around the mouth, nausea, vomiting, diarrhea, joint and muscle aches, headache, dizziness, muscle weakness, slow or fast heartbeat, low blood pressure, and being extremely sensitive to temperature. The symptoms usually go away in a few days, but in some cases, the neurologic symptoms (that is, symptoms like pain, numbness, tingling, etc.) may last much longer. These symptoms may go away and come back after many months, and it's thought that this return of symptoms may be somehow linked, in part, to eating or drinking alcohol, caffeine, nuts, and fish (even fish that don't contain poison). There is no proven treatment for the poison itself, but treatment may be needed for some of the symptoms. If you will be fishing in tropical areas and plan to eat what you catch, it would be a good idea to ask local health authorities about which fish in the area are safe to eat. At the end of this chapter is a list of the fish that are most likely to contain the poison. The list includes, for example, barracuda, amberjack, other large jacks, and large groupers and snappers. IT IS NOT A COMPLETE LIST, since it tells only which fish are *most likely* to contain the poison, from past experience. It's possible that other fish in warm-water (tropical) areas also could contain the poison. Waters near the U.S. where fish containing this poison have been found include those of South Florida, the Bahamas, the U.S. and British Virgin Islands, Puerto Rico, and Hawaii.

- **Symptoms**: Gastrointestinal symptoms include nausea, vomiting, and diarrhea. Neurological symptoms include perioral numbness and tingling (paresthesias), which may spread to the extremities; itching; arthralgia; myalgia; headache; acute sensitivity to temperature extremes; vertigo; and severe muscular weakness. Cardiovascular signs include arrhythmia, bradycardia or tachycardia, and hypotension.

- **Duration**: Symptoms of poisoning often subside within several days of onset. However, in severe cases, the neurological symptoms may persist from weeks to months. In a few isolated cases, neurological symptoms have persisted for several years, and, in other cases, patients who have recovered have experienced recurrence of neurological symptoms months to years afterwards. Such relapses are most often associated with consumption of fish (even non-toxic fish), alcohol, caffeine, or nuts.

- **Route of entry**: Oral.

- **Pathway**: Ciguatoxins are cyclic polyether compounds that bind to, and activate, voltage-sensitive sodium channels in excitable tissues.

3. Frequency

The relative frequency of ciguatera fish poisoning in the United States is not known; current estimates of the worldwide occurrence range from 50,000 to 500,000 cases per year. The disease has only recently become known to the general medical community, and there is a concern that the incidence is largely under-reported.

4. Sources

Marine finfish most commonly implicated in ciguatera fish poisoning include certain species of groupers, barracudas, snappers, jacks, mackerel, triggerfish, and others. Many warm-water marine fish species in tropical and subtropical waters may harbor ciguatera toxins. The occurrence of toxic fish is sporadic, and not all fish of a given species or from a given locality will be toxic. Areas that are noted for toxic fish in or near U.S. waters include South Florida, the Bahamas, the U.S. and British Virgin Islands, Puerto Rico, and Hawaii.

A list of fish species most likely to contain ciguatoxin is included at the end of this chapter. The list is not comprehensive, in that it contains only the names of the fish that, historically, are the *most likely* to contain the toxin.

5. Diagnosis

Clinical testing procedures are not presently available for the diagnosis of ciguatera in humans. Diagnosis is based entirely on signs, symptoms, and a history of having consumed fish from tropical or subtropical areas.

6. Target Populations

All humans are believed to be susceptible to ciguatera toxins. Populations in tropical / subtropical regions are most likely to be affected because of the frequency of exposure to toxic fish. However, the increasing per-capita consumption of fishery products, coupled with an increase in inter-regional transportation of seafood products, has expanded the geographic range of human poisonings.

7. Food Analysis

The ciguatera toxins can be recovered from toxic fish through time-consuming extraction and purification procedures. The mouse bioassay historically has been the accepted method of establishing toxicity of suspect fish. It has now been largely supplanted by in *vitro* (e.g., the cytotoxicity assay) and instrumental (e.g., LC-MS/MS) methods.

8. Examples of Outbreaks

MMWR 58(11): 2007 – Seven cases of ciguatera caused by consumption of amberjack were investigated by the Food and Drug Protection Division of the North Carolina Department of Agriculture and Consumer Services and the North Carolina Department of Health and Human Services.

MMWR 47(33):1998 – This report summarizes an investigation of this outbreak by the Texas Department of Health (TDH), which indicated that 17 crew members experienced ciguatera fish poisoning resulting from eating a contaminated barracuda.

MMWR 42(21):1993 – Twenty cases of ciguatera fish poisoning from consumption of amberjack were reported to the Florida Department of Health and Rehabilitative Services (HRS) in August and September 1991. This report summarizes the investigation of these cases by the Florida HRS.

MMWR 35(16):1986 – On October 29, 1985, the Epidemiology Division, Vermont Department of Health, learned of two persons with symptoms consistent with ciguatera fish poisoning. Both had eaten barracuda at a local restaurant on October 19.

MMWR 31(28):1982 – On March 6, 1982, the U.S. Coast Guard in Miami, Florida, received a request for medical assistance from an Italian freighter located in waters off Freeport, Bahamas. Numerous crew members were ill with nausea, vomiting, and muscle weakness and required medical evacuation for hospitalization and treatment. These findings were consistent with ciguatera fish poisoning.

Morbidity and Mortality Weekly Reports – For more information on recent outbreaks, check the Morbidity and Mortality Weekly Reports from the Centers for Disease Control and Prevention.

9. Other Resources

- Centers for Disease Control and Prevention ciguatera webpage
- Website for Project Caribcatch, a multi-institutional research project studying many facets of the ciguatera phenomenon.

10. Molecular Structures

Pacific ciguatoxin-1

Caribbean ciguatoxin-1

Some Potentially Ciguatoxic Fish Species

This list is ***NOT*** comprehensive; it includes only the names of the species that, historically, are ***most likely*** to be ciguatoxic. Other fish that do not appear on this list also may be ciguatoxic.

Caribbean, Atlantic, Gulf of Mexico	
Family name, *Latin name*	**Common name**
Balistidae	**Triggerfishes**
Balistes vetula	Queen triggerfish
Carangidae	**Jacks**
Caranx crysos	Blue runner
C. latus	Horse-eye jack
C. lugubris	Black jack
C. ruber	Bar jack
Carangoides bartholomaei	Yellow jack
Seriola dumerili	Greater amberjack
Labridae	**Wrasses**
Lachnolaimus maximus	Hogfish
Lutjanidae	**Snappers**
Lutjanus buccanella	Blackfin snapper
L. cyanopterus	Cubera snapper
L. griseus	Gray snapper
L. jocu	Dog snapper
Muraenidae	**Eels**
Gymnothorax funebris	Green moray eel
Scombridae	**Mackerel**
Scomberomorus cavalla	King mackerel, kingfish
Scomberomorus regalis	Cero mackerel
Serranidae	**Groupers, sea basses**
Mycteroperca bonaci	Black grouper
M. microlepis	Gag
M. phenax	Scamp
M. venenosa	Yellowfin grouper
Epinephelus adscensionis	Rock hind
E. guttatus	Red hind
Epinephelus (Dermatolepis) inermis	Marbled grouper

E. morio	Red grouper
Sphyraenidae	**Barracudas**
Sphyraena barracuda	Great barracuda

Pacific region

Family name, *Latin name*	Common name
Acanthuridae	**Surgeonfishes**
Ctenochaetus strigosus	Yellow eye tang, Kole
C. striatus	Striated (or striped) surgeonfish, bristle-tooth surgeon
Carangidae:	**Jacks**
Caranx ignobilis	Giant Trevally, Ulua
C. melampygus	Bluefin trevally, Black Ulua
Labridae	**Wrasses**
Cheilinus undulatus	Humphead wrasse
Lutjanidae:	**Snappers**
Lutjanus bohar	Twinspot snapper
L. gibbus	Paddletail
L. sebae	Emperor snapper
Aphareus spp.	Jobfishes
Aprion virescens	Green jobfish
Pristipomoides spp.	Jobfishes, snappers
Symphorus nematophorus	Chinaman fish, Chinaman snapper
Muraenidae	**Eels**
Gymnothorax (Lycodontis) javanicus	Giant moray
Scaridae	**Parrotfishes**
Scarus gibbus	Steepheaded parrotfish
Scombridae	**Mackerel**
Scomberomorus commerson	Narrow-barred spanish mackerel
Serranidae:	**Groupers, sea basses**
Cephalopholis argus	Peacock hind
C. miniata	Coral hind
Epinephelus fuscoguttatus	Brown-marbled grouper
E. lanceolatus	Giant grouper

Plectropomus spp.	Coral trout
Variola louti	Yellow-edged lyretail
Sphyraenidae	**Barracudas**
Sphyraena jello	Barracuda

Shellfish toxins (PSP, DSP, NSP, ASP, AZP)

1. Toxins

Shellfish poisoning is caused by a group of toxins produced by planktonic algae (dinoflagellates, in most cases) on which shellfish feed. The toxins are accumulated, and sometimes metabolized by, the shellfish. Numerous shellfish toxins have been described around the world; included here are toxins currently regulated by the FDA.

Paralytic shellfish poisoning (PSP) is caused by water-soluble alkaloid neurotoxins that are collectively referred to as saxitoxins or paralytic shellfish toxins (PSTs). To date 57 analogs have been identified, although not all are always present, and they vary greatly in overall toxicity. In addition to saxitoxin (the parent compound), monitoring laboratories typically analyze for approximately 12 other analogs that may contribute measurably to toxicity.

Diarrhetic shellfish poisoning (DSP) is caused by a group of lipid-soluble polyether toxins that includes okadaic acid, the dinophysistoxins, and a series of fatty acid esters of okadaic acid and the dinophysistoxins (collectively known as DSTs).

Neurotoxic shellfish poisoning (NSP) is caused by a group of lipid-soluble polyether toxins called brevetoxins. NSP-causing toxins in shellfish include intact algal brevetoxins and their metabolites (collectively known as NSTs).

Amnesic shellfish poisoning (ASP) is caused by the neurotoxin domoic acid (DA), a water-soluble, non-protein, excitatory amino acid. Isomers of domoic acid have been reported, but are less toxic than domoic acid itself.

For Consumers: A Snapshot

Algae are plant-like life-forms that float or move on their own in water. They vary in size from very small (microscopic) to very large (for example, seaweed, such as kelp). Some marine and freshwater algae make toxins (poisons). Many of the toxins that build up in shellfish – seafood such as oysters, clams, and mussels, to name a few – are made by a small type of algae called "dinoflagellates," which swim and have characteristics of both plants and animals. When shellfish eat these algae, the poisons can build up in the shellfish and sicken people who eat them. The kind of illness depends on the poison. Some can be deadly, like **paralytic shellfish poisoning (PSP)**. Others, like **diarrhetic shellfish poisoning** and **azaspiracid shellfish poisoning**, mostly cause symptoms like nausea, vomiting, diarrhea, and stomach pain. Besides these kinds of symptoms, some shellfish poisonings, like **neurotoxic shellfish poisoning**, also cause neurologic effects, such as tingling or numbness of lips and throat, dizziness, and muscle aches. In extreme cases, **amnesic shellfish poisoning** has resulted in severe neurologic disorders, such as loss of short-term memory, in some people. These poisons aren't destroyed by cooking, freezing, or other food preparation. This highlights the importance of FDA's seafood-safety programs, guidance to industry, and close working relationships with state regulators. For example, the levels of saxitoxins (which cause PSP) often become high in shellfish in New England waters at certain times of the year when the toxin-producing algae are present. When the level becomes too high for safety, state health agencies follow FDA guidance and ban shellfish harvesting, and PSP outbreaks from commercial products are very rare in the U.S.

Azaspiracid shellfish poisoning (AZP) is caused by the lipid-soluble toxin azaspiracid and several derivatives (AZAs). To date, more than 30 AZA analogs have been identified, with three analogs routinely monitored in shellfish.

2. Diseases

Human ingestion of contaminated shellfish results in a wide variety of symptoms, depending on the toxin(s) present, their concentrations in the shellfish, and the amount of contaminated shellfish consumed.

Note: The specific seafood with which each toxin generally is associated is included in this "Disease" section, to help readers link symptoms to potential sources. However, all shellfish (filter-feeding mollusks, as well as the carnivorous grazers that feed on these mollusks, such as whelk, snails, and, in some cases, even lobster and octopus) may become toxic in areas where the source algae are present. In most cases, the toxin has no effect on the shellfish itself, and how long each shellfish vector remains toxic depends on the individual species in question. Additionally, there are non-traditional and emerging vectors of these toxins that also are potentially toxic foods. One example is that pufferfish, which typically is associated with tetrodotoxin (see chapter on Tetrodotoxin), may also contain saxitoxin (e.g., puffers from coastal waters of Florida).

Paralytic Shellfish Poisoning

- ***Mortality:*** Death has been reported to occur as soon as 3 to 4 hours after the contaminated food has been consumed.

- ***Onset:*** Symptoms can generally occur within 30 minutes of consuming contaminated seafood, although reports have indicated that symptoms can even ensue within a few minutes, if high enough toxin concentrations are present.

- ***Symptoms and course of illness:*** Effects of PSP are predominantly neurologic and include tingling of the lips, mouth, and tongue; numbness of extremities; paresthesias; weakness; ataxia; floating/dissociative feelings; nausea; shortness of breath; dizziness; vomiting; headache; and respiratory paralysis.

  Medical treatment consists of providing respiratory support, and fluid therapy can be used to facilitate toxin excretion. For patients surviving 24 hours, with or without respiratory support, the prognosis is considered good, with no lasting side effects. In fatal cases, death is typically due to asphyxiation. In unusual cases, death may occur from cardiovascular collapse, despite respiratory support, because of the weak hypotensive action of the toxin.

- ***Food Sources:*** PSP generally is associated with bivalves, such as mussels, clams, cockles, oysters, and scallops (excluding the scallop adductor muscle).

Diarrhetic Shellfish Poisoning

- ***Mortality:*** This disease generally is not life-threatening.

- ***Onset:*** Onset of the disease, depending on the dose of toxin ingested, may be as little as 30 minutes to 3 hours.

- ***Symptoms and course of illness:*** DSP is primarily observed as a generally mild gastrointestinal disorder; i.e., nausea, vomiting, diarrhea, and abdominal pain, accompanied by chills, headache, and fever. Symptoms may last as long as 2 to 3 days, with no chronic effects.

- ***Food Sources:*** DSP generally is associated with mussels, oysters, and scallops.

Neurotoxic Shellfish Poisoning

- ***Mortality:*** No fatalities have been reported.

- ***Onset:*** Onset of this disease occurs within a few minutes to a few hours.

- ***Symptoms and course of illness:*** Both gastrointestinal and neurologic symptoms characterize NSP, including tingling and numbness of lips, tongue, and throat; muscular aches; dizziness; diarrhea; and vomiting. Duration is fairly short, from a few hours to several days. Recovery is complete, with few after-effects.

- ***Food Sources:*** NSP generally is associated with oysters and clams harvested along the Florida coast and the Gulf of Mexico. In 1992 / 1993, NSP was linked to shellfish harvested from New Zealand.

Amnesic Shellfish Poisoning

- ***Mortality:*** All fatalities, to date, have involved elderly patients.

- ***Onset:*** The toxicosis is characterized by onset of gastrointestinal symptoms within 24 hours; neurologic symptoms occur within 48 hours.

- ***Symptoms and course of illness:*** ASP is characterized by gastrointestinal disorders (vomiting, diarrhea, abdominal pain) and neurological problems (confusion, short-term memory loss, disorientation, seizure, coma). Human clinical signs of domoic acid toxicity are reported as mild gastrointestinal symptoms, from an oral dose of 0.9-2.0 mg domoic acid (DA)/kg body weight. Neurologic effects, such as seizure and disorientation, are reported from an oral dose of 1.9-4.2 mg DA/kg body weight. The toxicosis is particularly serious in elderly patients, and includes symptoms reminiscent of Alzheimer's disease.

- ***Food Sources:*** ASP generally is associated with mussels. Other taxa of interest include scallops, razor clams, market squid, and anchovy.

Azaspiracid Shellfish Poisoning

- ***Mortality:*** No known fatalities to date.

- ***Onset:*** Symptoms appear in humans within hours of eating AZA-contaminated shellfish.

- ***Symptoms and course of illness:*** Symptoms are predominantly gastrointestinal disturbances resembling those of diarrhetic shellfish poisoning and include nausea, vomiting, stomach cramps, and diarrhea. Illness is self-limiting, with symptoms lasting 2 or 3 days.

- ***Food Sources:*** AZAs have been detected in mussels, oysters, scallops, clams, cockles, and crabs.

3. Diagnosis

Diagnosis of shellfish poisoning is based entirely on observed symptomatology and recent dietary history.

4. Frequency

Good statistical data on the occurrence and severity of shellfish poisoning are largely unavailable, which undoubtedly reflects the inability to measure the true incidence of the disease. Cases are frequently misdiagnosed and, in general, infrequently reported. The proliferation (sometimes referred to as "blooms") of the toxin-producing algae and subsequent toxin events or outbreaks of illness appear to be increasing around the world. To combat this, seafood monitoring programs enforce harvesting bans when toxins exceed their respective regulatory action levels. In many countries, including the United States, this has resulted in protection of public health. Additional information on the frequency and severity of outbreaks for the various shellfish toxins around the world can be found in the Resources section, below.

5. Target Populations

All humans are susceptible to shellfish poisoning. A disproportionate number of shellfish-poisoning cases occur among (1) tourists or others who are not native to the location where the toxic shellfish are harvested and (2) fishermen and recreational harvesters. This may be due to disregard for either official quarantines or traditions of safe consumption.

6. Food Analysis

According to the 4th edition of the FDA Fish and Fisheries Products Hazards and Controls Guidance, regulatory action levels for the shellfish toxins are as follows:

- **PSP** – 0.8 ppm (80 µg/100 g) saxitoxin equivalents
- **NSP** – 0.8 ppm (20 mouse units/100 g) brevetoxin-2 equivalents
- **DSP** – 0.16 ppm total okadaic acid equivalents (i.e., combined free okadaic acid, dinophysistoxins, acyl-esters of okadaic acid and dinophysistoxins)
- **ASP** – 20 ppm domoic acid (except in the viscera of Dungeness crab, for which the action level is 30 ppm)
- **AZP** – 0.16 ppm azaspiracid 1 equivalent

The mouse bioassay historically has been the most universally applied technique for examining shellfish toxins. Other bioassay procedures have been developed and are becoming more generally applied. In recent years, considerable effort has been applied to development of chemical analyses to replace or provide alternatives to *in-vivo* (live animal) bioassays. Examples are included below.

> **Paralytic Shellfish Poisoning (PSP):** The mouse bioassay is still the most widely accepted detection method for the **saxitoxins** around the world and has been shown to

adequately protect the public's health. However, a pre-column oxidation, high-performance liquid chromatography (HPLC) with fluorescence detection (FD) method has received AOAC approval and has become a regulatory tool in some countries. This method is the only one currently listed for **saxitoxins** in the Codex Alimentarius "Standard for Live and Raw Bivalve Molluscs." In 2009 the Interstate Shellfish Sanitation Conference approved a post-column oxidation HPLC-FD approach as a Type IV NSSP (National Shellfish Sanitation Program) method, making it the newest regulatory method available for **PSP** toxins in the U.S. This method also gained AOAC approval in 2011. The receptor binding assay, a competition assay whereby radiolabeled saxitoxin competes with unlabeled saxitoxin for a finite number of available receptor sites as a measure of native saxitoxin concentrations in a sample, was also approved as an official AOAC method in 2011.

Diarrhetic Shellfish Poisoning (DSP): Until recently, **DSP** toxins were not monitored in the U.S. In other parts of the world, a mouse bioassay was used to assess **diarrhetic shellfish toxins (DST)** presence, but this assay was neither sensitive nor specific enough to adequately protect public health. The dose-survival times for the **DSP** toxins in the mouse assay fluctuate considerably, and fatty acids and other co-occurring non-diarrhetic compounds interfere with the assay, giving false-positive results. Consequently, a suckling mouse assay that measures fluid accumulation after injection of a shellfish extract was developed and used for control of DSP. Due to a mandate to eliminate *in-vivo* mouse assays for lipophilic toxins in the European Union (EU), numerous alternative methods are in various stages of development and validation around the world. These include liquid chromatography / mass spectrometry (LC/MS), antibody-based commercial kits, and several *in-vitro* bioactivity assays based on phosphatase inhibition.

Neurotoxic Shellfish Poisoning (NSP): Toxicity of shellfish exposed to the dinoflagellate *Karenia brevis* has been historically assessed by mouse bioassay in the U.S. Mouse bioassay is not very specific for **NSP** toxins. Thus, efforts are underway to validate *in-vitro* methods for detection of **brevetoxins** in shellfish. For example, rapid, sensitive ELISA test kits already are commercially available for this purpose. Biomarkers of **brevetoxin** contamination in shellfish have been identified by using LC/MS. Structural confirmation of these metabolites and brevetoxins in shellfish can be made by LC/MS, a method that offers high sensitivity and specificity.

Amnesic Shellfish Poisoning (ASP): The mouse bioassay for **domoic acid** is not sufficiently sensitive and does not provide a reliable estimate of potency. The most accepted regulatory method for detecting **domoic acid** in seafood is a reversed-phase HPLC method with ultraviolet (UV) detection. There is also an AOAC approved ELISA for the detection of **domoic acid**.

Azaspiracid Shellfish Poisoning (AZP): AZAs are not routinely monitored in shellfish harvested in the U.S., but, in the EU, the mouse bioassay has been used. As for many of the lipophilic toxins, the mouse assay is not adequately sensitive or specific for public-health purposes. *In-vitro* assays and analytical methods are now available to assess the toxicity of AZA-contaminated shellfish and to confirm the presence of AZA analogs in shellfish. These methods are in various stages of validation for regulatory use around the world. LC/MS is used as a confirmatory method for AZA, providing unambiguous structural confirmation of AZA analogs in shellfish samples.

7. Examples of Outbreaks

PSP – Despite widespread PSP closures, poisoning events still occur and are generally associated with recreational harvest. For example, in July 2007, a lobster fisherman harvested mussels from a floating barrel off Jonesport, ME (an area that was currently open to shellfish harvesting), and he and his family ate them for dinner. All four consumers became ill with PSP symptoms, and three of them were admitted to the hospital. It was apparent that the barrel of mussels had originated further up the coast in an area that had been banned to commercial harvest.

DSP – Although there have been numerous outbreaks of diarrhetic shellfish poisoning around the world, until recently there were no confirmed cases of DSP in the U.S. that were due to domestically harvested shellfish. However, in 2008, a large portion of the Texas Gulf Coast was closed to the harvesting of oysters due to the presence of okadaic acid in excess of the FDA guidance level. Although no illnesses were reported, these were the first closures in the U.S. due to confirmed toxins. In 2011, approximately 60 illnesses occurred in British Columbia, Canada, and 3 illnesses occurred in Washington State due to consumption of DSP-contaminated mussels. Subsequent harvesting closures and product recalls were issued.

NSP – Until NSP toxins were implicated in more than 180 human illnesses in New Zealand, in 1992/1993, NSP was considered to be an issue only in the U.S. Outbreaks of NSP are rare where programs for monitoring *K. brevis* blooms and shellfish toxicity are implemented. An NSP outbreak involving 48 individuals occurred in North Carolina, in 1987. A series of NSP cases occurred along the southwest coast of Florida, in 2006, after people consumed recreationally-harvested clams from waters unapproved for shellfish harvesting.

ASP - The first human domoic acid poisoning events were reported in 1987, in Canada. While domoic acid exposure still exists, there have been no documented ASP cases since 1987, following implementation of effective seafood toxin-monitoring programs.

AZP – There have been no confirmed cases of AZP in the U.S. from domestically harvested product. Examples from around the world include: (1) Several AZP intoxications (20 to 24) were reported in Ireland, in 1997, following consumption of mussels harvested from Arranmore Island. (2) An AZP outbreak involving 10 people was reported in Italy, after they consumed contaminated mussels produced in Clew Bay, Ireland. (3) In 1998, in France, 20 to 30 AZP illnesses were attributed to scallops that originated in Ireland. (4) In 2008, the first recognized outbreak of AZP in the U.S. was reported, but was associated with a mussel product imported from Ireland.

CDC/MMWR: Various Shellfish-Associated Toxins provides a list of Morbidity and Mortality Weekly Reports related to these toxins.

NIH/PubMed: Various Shellfish-Associated Toxins provides a list of research abstracts in the National Library of Medicine's MEDLINE database.

8. Resources

Food and Agriculture Organization of the United Nations Paper 80: Marine Biotoxins

Paralytic Shellfish Poisoning

Diarrheic Shellfish Poisoning

Neurotoxic Shellfish Poisoning

Additional Resources [open access]

Twiner MJ, Rehmann N, Hess P, Doucette GJ. Azaspiracid Shellfish Poisoning: A Review on the Chemistry, Ecology, and Toxicology with an Emphasis on Human Health Impacts. *Mar. Drugs* 2008, *6*, 39-72.

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Wiese M, D'Agostino PM, Mihali TK, Moffitt MC, Neilan BA. Neurotoxic Alkaloids: Saxitoxin and Its Analogs. *Mar. Drugs* 2010, *8*, 2185-2211.

Deeds JR, Landsberg JH, Etheridge SM, Pitcher GC, Longan SW. Non-Traditional Vectors for Paralytic Shellfish Poisoning. *Mar. Drugs* **2008**, *6*, 308-348.

Pulido OM. Domoic Acid Toxicologic Pathology: A Review. *Mar. Drugs* 2008, *6*, 180-219.

9. Molecular Structure

Azaspiracid (AZA analogs produced by the dinoflagellate Azadinium spinosum: AZA1, AZA2, and an isomer of AZA2. Major analogs found in shellfish are AZA1, AZA2, and AZA3.)

Brevetoxin and related compounds

Saxitoxin and related compounds

Okadaic acid and related compounds

Domoic acid

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Scombrotoxin

For Consumers: A Snapshot

Scombrotoxin is a combination of substances that form when certain fish aren't properly refrigerated before being processed or cooked. One of the substances is histamine, which causes, for example, blood vessels to dilate and intestinal muscle to contract. Examples of fish that can form the toxin if they start to spoil include tuna, mahimahi, bluefish, sardines, mackerel, amberjack, and anchovies. The fish might not look or smell bad, but can cause illness. In the U.S., it's one of the most common illnesses caused by seafood. The symptoms, which should be treated with antihistamines by a health professional, usually are mild and start within minutes or hours after eating. They may include tingling or burning of the mouth or throat, rash or hives, low blood pressure, itching, headache, dizziness, nausea, vomiting, diarrhea, fluttery heartbeat, and trouble breathing. The symptoms usually go away in a few hours, but may go on for days, in severe cases. People who are on some medications, including tuberculosis drugs, or who have other medical conditions, are more likely to have severe reactions. Those are rare, but may include serious heart and lung problems. Be sure to tell your doctor if you ate fish, and when, to help with diagnosis. Cooking, freezing, and canning won't "get rid" of this toxin after it has formed. The best prevention is to try to keep it from forming in the first place, by keeping fish refrigerated at 40°F or lower.

1. Toxin

Scombrotoxin is a combination of substances, histamine prominent among them. Histamine is produced during decomposition of fish, when decarboxylase enzymes made by bacteria that inhabit (but do not sicken) the fish interact with the fish's naturally occurring histidine, resulting in histamine formation. Other vasoactive biogenic amines resulting from decomposition of the fish, such as putrescine and cadaverine, also are thought to be components of scombrotoxin. Time / temperature abuse of scombrotoxin-forming fish (e.g., tuna and mahi-mahi) create conditions that promote formation of the toxin. Scombrotoxin poisoning is closely linked to the accumulation of histamine in these fish.

FDA has established regulatory guidelines that consider fish containing histamine at 50 ppm or greater to be in a state of decomposition and fish containing histamine at 500 ppm or greater to be a public health hazard. The European Union issued Council Directive (91/493/EEC) in 1991, which states that when 9 samples taken from a lot of fish are analyzed for histamine, the mean value must not exceed 100 ppm; two samples may have a value of more than

100 ppm, but less than 200 ppm; and no sample may have a value exceeding 200 ppm.

2. Disease

The disease caused by scombrotoxin is called scombrotoxin poisoning or histamine poisoning. Treatment with antihistamine drugs is warranted when scombrotoxin poisoning is suspected.

- **Mortality**: No deaths have been confirmed to have resulted from scombrotoxin poisoning.
- **Dose:** In most cases, histamine levels in illness-causing (scombrotoxic) fish have exceeded 200 ppm, often above 500 ppm. However, there is some evidence that other biogenic amines also may play a role in the illness.

- **Onset**: The onset of intoxication symptoms is rapid, ranging from minutes to a few hours after consumption.

- **Disease / complications:** Severe reactions (e.g., cardiac and respiratory complications) occur rarely, but people with pre-existing conditions may be susceptible. People on certain medications, including the anti-tuberculosis drug isoniazid, are at increased risk for severe reactions.

- **Symptoms**: Symptoms of scombrotoxin poisoning include tingling or burning in or around the mouth or throat, rash or hives, drop in blood pressure, headache, dizziness, itching of the skin, nausea, vomiting, diarrhea, asthmatic-like constriction of air passage, heart palpitation, and respiratory distress.

- **Duration**: The duration of the illness is relatively short, with symptoms commonly lasting several hours, but, in some cases, adverse effects may persist for several days.

- **Route of entry**: Oral.

- **Pathway**: In humans, histamine exerts its effects on the cardiovascular system by causing blood-vessel dilation, which results in flushing, headache, and hypotension. It increases heart rate and contraction strength, leading to heart palpitations, and induces intestinal smooth-muscle contraction, causing abdominal cramps, vomiting, and diarrhea. Histamine also stimulates motor and sensory neurons, which may account for burning sensations and itching associated with scombrotoxin poisoning. Other biogenic amines, such as putrescine and cadaverine, may potentiate scombrotoxin poisoning by interfering with the enzymes necessary to metabolize histamine in the human body.

3. Frequency

Scombrotoxin poisoning is one of the most common forms of fish poisoning in the United States. From 1990 to 2007, outbreaks of scombrotoxin poisoning numbered 379 and involved 1,726 people, per reports to the Centers for Disease Control and Prevention (CDC). However, the actual number of outbreaks is believed to be far greater than that reported.

4. Sources

Fishery products that have been implicated in scombrotoxin poisoning include tuna, mahi-mahi, bluefish, sardines, mackerel, amberjack, anchovies, and others. Scombrotoxin-forming fish are commonly distributed as fresh, frozen, or processed products and may be consumed in a myriad of product forms. Distribution of the toxin within an individual fish or between cans in a case lot can be uneven, with some sections of a product capable of causing illnesses and others not. Cooking, canning, and freezing do not reduce the toxic effects. Common sensory examination by the consumer cannot ensure the absence or presence of the toxin. Chemical analysis is a reliable test for evaluating a suspect fishery product. Histamine also may be produced in other foods, such as cheese and sauerkraut, which also has resulted in toxic effects in humans.

5. Diagnosis

Diagnosis of the illness is usually based on the patient's symptoms, time of onset, and the effect of treatment with antihistamine medication. The suspected food should be collected; rapidly chilled or, preferably, frozen; and transported to the appropriate laboratory for histamine

analyses. Elevated levels of histamine in food suspected of causing scombrotoxin poisoning aid in confirming a diagnosis.

6. Target Populations

All humans are susceptible to scombrotoxin poisoning; however, as noted, the commonly mild symptoms can be more severe for individuals taking some medications, such as the anti-tuberculosis drug isoniazid. Because of the worldwide network for harvesting, processing, and distributing fishery products, the impact of the problem is not limited to specific geographic areas or consumption patterns.

7. Food Analysis

The official method (AOAC 977.13) for histamine analysis in seafood employs a simple alcoholic extraction and quantitation by fluorescence spectroscopy. Putrescine and cadaverine can be analyzed by AOAC Official Method 996.07. Several other analytical procedures to quantify biogenic amines have been published in the literature.

8. Examples of Outbreaks

- CDC/MMWR: Scombrotoxin provides a list of Morbidity and Mortality Weekly Reports at CDC relating to this toxin.
- NIH/PubMed: Scombrotoxin provides a list of relevant research abstracts contained in the National Library of Medicine's MEDLINE database.
- Agricola: Scombrotoxin provides a list of relevant research abstracts contained in the National Agricultural Library database.
- For more information on recent outbreaks, see the Morbidity and Mortality Weekly Reports from CDC.

9. Resources

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10. Molecular Structural Data:

Histamine produced by growth of certain bacteria and the subsequent action of their decarboxylase enzymes on histidine.

Tetrodotoxin

1. Toxin

Tetrodotoxin (TTX) and related compounds (e.g. 4,9-anhydroTTX, 4-*epi*TTX, 11-deoxyTTX, tetrodonic acid)

Poisoning from consumption of members of the family tetraodontidae (pufferfish) – i.e., pufferfish poisoning – is one of the most dangerous intoxications from marine species. There are approximately 185 species of pufferfish worldwide, and they occur in both freshwater and marine environments. Several of these species are consumed throughout the world, particularly in the Indo-Pacific region, such as Japan, where pufferfish hold great cultural significance. In several species, the gonads (mainly ovary), liver, intestines, and skin can contain levels of tetrodotoxin sufficient to produce rapid death. In a few species, the flesh naturally contains enough toxin to be lethal, if consumed.

Among the numerous pufferfish species, total toxicity, as well as toxin distribution among different organs within individual fish, can vary greatly. However, toxin presence and distribution does appear to be fairly consistent within a given species. As an example, the table at the end of this chapter provides the popular and scientific names for 22 species of pufferfish consumed in Japan, including which parts are

For Consumers: A Snapshot

In some parts of the world, especially Japan, pufferfish (also called "fugu" or "blowfish") are thought of as a delicacy – even though they contain a poison that's deadly to humans, if the fish aren't prepared by a highly trained expert. In some types of pufferfish, some organs, like the liver and skin, contain the poison, which is called tetrodotoxin. If the chef or trained cutter doesn't cut the fish in *exactly* the right way, the poison may get into the meat of the fish, and the person who eats it may become ill or even die without immediate medical treatment. In mild cases of pufferfish poisoning, the person who eats it may get numbness and tingling in the lips, arms, and legs, and may feel light-headed. In severe cases, death is from suffocation – often awake until the end – because of paralyzed breathing muscles. There are many types (species) of pufferfish, and in most of them, only the organs, not the meat, naturally contain the poison. Other types don't contain any of the poison at all, like the puffer from the mid-Atlantic waters of the U.S., called "northern puffer." This type of pufferfish used to be sold as "sea squab," but today restaurants sell it under other names, such as "sugar toad." On the other hand, a few types of pufferfish naturally have large amounts of the poison in their meat (not just the organs), and it's never safe to eat them, no matter who prepares them. After a fish has been cleaned and processed (for example, turned into fillets or fish cakes), it can be hard to tell what kind it is. Because of this, the FDA allows only one type of puffer (*Takifugu rubripes*, also called torafugu or tiger puffer) to be imported from Japan. Only certain parts are allowed, and it has to be prepared by trained fish cutters before it's imported. It's sold only to restaurants belonging to a specific association. Because of these strict safety limitations, the availability of this pufferfish often is limited, and it's often expensive. Several times, the FDA has stopped illegally imported shipments of pufferfish. In some cases, unsafe importers have tried to get puffers into the country labeled as different fish. Puffer – the dangerous kind – falsely labeled as monkfish was imported from China in 2007 and sickened people who had eaten bok go jim (blowfish casserole) or bok jiri (blowfish stew) in restaurants. "Bok" is a Korean word for "puffer." In Illinois, home-made puffer soup made from bok, from a local ethnic market, caused illness. The message to take away from all this is that if you choose to eat pufferfish, eat only those from sources known to be safe. (Also see the box called "DNA Barcoding" at the end of the Gempylotoxin chapter of this book.)

considered edible (non-toxic). This list is not comprehensive for all species of pufferfish consumed around the world and is not a recommended list of edible species for consumers in the United States. In Japan, the Ministry of Health, Labour, and Welfare provides strict guidance and regulation for the harvesting and consumption of pufferfish. Under this guidance, the flesh for many of these species is considered safe to consume, if prepared properly by a trained expert so as not to contaminate the fish's flesh with toxin from its other tissues. Today, most poisonings in Japan result from consumption of home-prepared dishes from pufferfish that have been caught recreationally. Authorities in Japan prohibit the use of all viscera from all species of pufferfish, especially the liver and ovaries, for use as food.

Regulations vary or do not exist in many of the other Indo-Pacific societies that consume pufferfish. For example, in Taiwan, two species of marine pufferfish, Kurosabafugu (*Lagocephalus gloveri*) and Shirosabafugu (*L. wheeleri*), are considered safe for consumption and are used to produce dried fish fillets and fish balls. A closely related species, *Lagocephalus lunaris*, is one of the only species known to contain dangerously high levels of TTX naturally in its flesh, in addition to its viscera. This species has been associated with illness not only in Taiwan, where it has been used accidentally as dried fish fillets, but also in other countries, from which it has been exported under false names, such as monkfish and anglerfish.

Tetrodotoxin also has been isolated from other animal species, including newts, tropical gobies, frogs, the blue-ringed octopus, starfish, trumpet shells (gastropods), horseshoe crabs, and xanthid crabs. Although occasionally consumed and associated with illness in other parts of the world, none of these species are imported into the U.S. for human consumption. Blue-ringed octopi are unique in that they inject TTX when they bite their prey, making the poison also a venom, and several intoxications have occurred through accidental contact by divers and home-aquarium hobbyists.

These toxins are both heat- and acid-stable. They are not destroyed by cooking or freezing.

2. Disease

- **Mortality**: Death is from respiratory-muscle paralysis and usually occurs within 4 to 6 hours, with a known range of about 20 minutes to 8 hours.

- **Lethal dose**: The minimum lethal dose in humans is estimated to be 2 to 3 mg (1/500 of a gram).

- **Onset**: The first symptom of intoxication is a slight numbness of the lips and tongue, typically appearing between 20 minutes to 3 hours after ingestion, depending on the ingested dose. With higher doses, symptoms can start within minutes.

- **Illness / complications**: Tetrodotoxin acts on both the central and peripheral nervous systems. After the initial slight oral numbness, the next symptom is increasing paraesthesia in the face and extremities, which may be followed by sensations of lightness or floating. Headache, epigastric pain, nausea, diarrhea, and/or vomiting may occur. Occasionally, some reeling or difficulty in walking may occur.

  The second stage of the intoxication includes progressive paralysis. Many victims are unable to move; even sitting may be difficult. There is increasing respiratory distress. Speech is affected, and the victim usually exhibits dyspnea, cyanosis, and hypotension. Paralysis increases, and convulsions, mental impairment, and cardiac arrhythmia may

occur. The victim, although completely paralyzed, may be conscious and, in some cases, completely lucid until shortly before death.

There is no antidote for TTX poisoning, and treatment is symptomatic and supportive. Patients who receive ventilatory support recover fully, in most cases.

- **Symptoms**: See "Illness / complications," above.
- **Duration**: It is generally considered that if victims survive the initial 24 hours, they are expected to recover fully. It is known that TTX is cleared from the human body relatively quickly (in days) through the urine. Other symptoms, such as muscle weakness, can persist longer. No chronic effects have been reported.
- **Route of entry**: Oral.
- **Pathway**: Tetrodotoxin acts directly on voltage-activated sodium channels in nerve tissue. Toxin binding to the channel blocks the diffusion of sodium ions, preventing depolarization and propagation of action potentials. All of the observed toxicity is secondary to action-potential blockage.

3. Frequency

Only a few cases of intoxication from TTX have been reported in the U.S., and only from consumption of pufferfish. In Japan, however, 1,032 cases of pufferfish poisoning (PFP) were reported from 1965 through 2007, with 211 fatalities. In 1983, the Japanese Ministry of Health, Labour, and Welfare enacted guidance for pufferfish harvest and consumption, thereby greatly reducing the number of illnesses and mortalities from commercial product. Between 2002 and 2006, however, 116 incidents of PFP, with 223 individuals intoxicated and 13 mortalities, were reported, suggesting that problems still occur. Most of these illnesses were from home-prepared meals made from recreationally harvested fish.

Data for other Indo-Pacific countries are not easily available, but fatalities have been reported from consumption of pufferfish, gobies, trumpet shells, and xanthid crabs. It should be noted that certain pufferfish and xanthid crabs have been shown to also contain additional, potentially lethal toxins, such as saxitoxin and palytoxin (see sidebar).

4. Sources

The metabolic source of TTX is uncertain. No algal source has been identified, and TTX was originally assumed to be a metabolic product of the host. However, TTX has now been found throughout marine food webs, including high concentrations in some benthic invertebrates. More recently, reports of the production of TTX / anhydrotetrodotoxin by several bacterial species, including strains of the family Vibrionaceae, *Shewanella* spp., and *Alteromonas tetraodonis*, point toward a possible bacterial origin of this family of toxins, although high and consistent production of TTX and related compounds in laboratory isolates has yet to be achieved. Traditionally toxic species of pufferfish cultured from birth, in captivity and removed from environmental sources of TTX, have been found to remain non-toxic. Through subsequent exposure of these fish to TTX in their diet, it has been shown that these species can rapidly accumulate the toxin and distribute it to various internal organs, giving further evidence of a food-chain source of TTX and a metabolic predisposition toward accumulation of these toxins in certain pufferfish species.

Reports of PFP in the U.S. from commercial product are rare. In 1996, several people were intoxicated by product hand-carried from Japan. In 2007, several PFP cases were linked to product illegally imported as monkfish. In this case, the product in question was believed to be *L. lunaris*, one of the only species known to contain dangerous levels of toxin naturally in its flesh, making it unfit for consumption, regardless of preparation method or training of the preparer.

There are strict regulations on importation of pufferfish into the U.S. Only muscle, skin, and testicles from a single species (*Takifugu rubripes*, a.k.a. tiger puffer or torafugu) are allowed entry into the U.S. from Japan. These products must be processed in a certified facility by trained personnel and certified as safe for consumption by the Japanese government. Any pufferfish products imported outside the guidelines of this agreement are subject to detention without physical examination, under FDA Import Alert #16-20.

As many as 19 species of pufferfish occur in U.S. waters, many of which contain TTX. Over the past 50 years, sporadic and isolated cases of pufferfish poisoning, including a few fatalities, involved pufferfish from the Atlantic Ocean, Gulf of Mexico, and Gulf of California. There have been no confirmed cases of poisoning from the northern pufferfish, *Sphoeroides maculatus*, which was once harvested on the U.S. east coast and marketed as "sea squab." The northern pufferfish is known not to contain TTX.

Due to the fact that imported pufferfish are limited to a single species (*T. rubripes*) processed and certified as safe prior to importation, the domestic puffer (sea squab) fishery targets a non-toxic species, and the U.S. does not import other species known to contain TTX (i.e. trumpet shells, xanthid crabs, etc.) for food. The FDA makes no recommendations for control of TTX in seafood in its Fish and Fisheries Products Hazards and Controls Guidance. However, due to recent issues with the illegal importation of misbranded Asian pufferfish and the recent appearance of saxitoxin in east-coast Florida southern pufferfish (*Sphoeroides nephelus*) – described in the sidebar below – FDA advises consumers who choose to consume pufferfish to consume only those from known safe sources.

5. Diagnosis

The diagnosis of PFP is based on the observed symptomatology and recent dietary history. A case definition is available from the Centers for Disease Control and Prevention.

6. Target populations

All humans are susceptible to TTX poisoning. This toxicosis may be avoided by not consuming pufferfish or other animal species containing TTX. In the U.S., most other animal species known to contain TTX are not usually consumed by humans. Poisoning from TTX is of major public health concern primarily in Japan and other Indo-Pacific countries, where "fugu" is a traditional delicacy. In Japan, it is prepared and sold in special restaurants, where trained and licensed individuals carefully remove the viscera to reduce the danger of poisoning. Due to its import restrictions and high value, there is potential for intentional mislabeling and illegal importation, particularly of prepared, frozen fish products. Several firms have been placed on the FDA Import Alert list for species misbranding and illegal importation of pufferfish.

7. Food Analysis

The mouse bioassay for paralytic shellfish poisoning (PSP) can be used to monitor TTX in seafood products. An HPLC method with post-column reaction with alkali and fluorescence has

been developed to determine TTX and its associated toxins. The alkali degradation products can also be confirmed as their trimethylsilyl derivatives, by gas chromatography. Mass spectrometry methods have been developed and show good sensitivity and selectivity. Antibody- and receptor-based methods are also available. To date, none of these chemical methods have been validated for regulatory compliance.

8. Examples of Outbreaks

On April 29, 1996, three cases of TTX poisoning occurred among chefs in California who shared contaminated fugu (pufferfish) brought from Japan by a co-worker as a prepackaged, ready-to-eat product. The quantity eaten by each person was minimal, ranging from approximately ¼ to 1½ oz. Onset of symptoms began approximately 3 to 20 minutes after ingestion, and all three chefs were transported by ambulance to a local emergency department.

Three deaths were reported in Italy, in 1977, following consumption of frozen pufferfish imported from Taiwan and mislabeled as angler fish.

In 2007, it was reported that fish sellers in Thailand were selling meat from a highly poisonous species of pufferfish labeled as salmon. This practice led to the death of 15 people over a 3-year period.

In 2007, four separate incidents of TTX poisoning occurred in California, Illinois, and New Jersey, all linked to the pufferfish species *L. lunaris* imported from China, illegally invoiced as monkfish to avoid import restrictions. For several of the poisonings, the product in question was being sold as "bok," a Korean term for pufferfish.

The sidebar below describes 28 cases of PFP, from consumption of southern pufferfish, (*Sphoeroides nephelus*) that occurred on the U.S. east coast between 2002 to 2004, believed to be due not to TTX, but from accumulation of saxitoxins.

For more information on recent outbreaks in the U.S., see the Morbidity and Mortality Weekly Reports (MMWR) from CDC.

9. Resources

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12. Molecular Structure

Tetrodotoxin

13. Examples of puffer species considered safe for consumption in Japan*, including which parts are considered edible.

Japanese Common Name	Scientific Name	Edible Part		
		Muscle	Skin	Male Gonad
Kasafugu	*Takifugu niphobles*	Yes	No	No
Komonfugu	*T. poecilonotus*	Yes	No	No
Higanfugu	*T. pardalis*	Yes	No	No
Shousaifugu	*T. snyderi*	Yes	No	Yes
Mafugu	*T. porphyreus*	Yes	No	Yes
Karasu	*T. chinensis*	Yes	Yes	Yes
Mefugu	*T. obscurus*	Yes	No	Yes
Akamefugu	*T. chrysops*	Yes	No	Yes
Nashifugu	*T. vermicularis*	Yes	No	No
Torafugu	*T. rubripes*	Yes	Yes	Yes
Shimafugu	*T. xanthopterus*	Yes	Yes	Yes
Gomafugu	*T. stictonotus*	Yes	No	Yes
Sansaifugu	*T. flavidus*	Yes	No	No
Kanafugu	*Lagocephalus inermis*	Yes	Yes	Yes
Shirosabafugu	*L. wheeleri*	Yes	Yes	Yes
Kurosabafugu	*L. gloveri*	Yes	Yes	Yes
Yoritofugu	*Sphoeroides pachygaster*	Yes	Yes	Yes
Ishigakifugu	*Chilomycterus reticulatus*	Yes	Yes	Yes
Harisenbon	*Diodon holocanthus*	Yes	Yes	Yes
Hitozuraharisenbon	*D. liturosus*	Yes	Yes	Yes
Nezumifugu	*D. hystrix*	Yes	Yes	Yes
Hakofugu	*Ostraction immaculatum*	Yes	No	Yes

* This does ***not*** imply that FDA encourages consumption of other species.
People who choose to consume any species of toxic pufferfish do so at their own risk.

A Different Toxin in Some Pufferfish

State bans harvesting in certain counties

Beginning suddenly in 2002 and extending to 2004, there were 28 cases of pufferfish poisoning from New Jersey to Florida, all of which were linked to southern pufferfish (*Sphoeroides nephelus*) harvested from the Indian River Lagoon system on Florida's east coast. (Only one case was from commercially harvested product.) However, these poisonings were shown to be due *not* to the usual form of pufferfish poisoning (tetrodotoxin), but, instead, to paralytic shellfish poisoning (saxitoxin and its derivatives, addressed separately in the chapter on shellfish poisoning). Saxitoxin and tetrodotoxin have nearly identical pharmacology and generate similar symptoms. The initial source of saxitoxins in this lagoon system is the marine algae *Pyrodinium bahamense*, which is concentrated by small bivalve mollusks, which, in turn, are consumed by puffers, in whose flesh the saxitoxins accumulate. Since the saxitoxin is in the puffers' flesh, no method of preparation can make the puffers from this region safe to consume. Florida southern puffers from outside the Indian Lagoon system have been shown to contain substantially less saxitoxin. The additional co-occurring puffer species *Sphoeroides testudineus* (checkered puffers) and *S. spengleri* (bandtail puffers) have been shown to contain both saxitoxin in their flesh and tetrodotoxin in their internal organs. Since 2004, Florida has banned the harvesting of all puffer species in the east coast counties of Volusia, Brevard, Indian River, St. Lucie, and Martin, due to the presence of saxitoxin in puffer flesh. Updates on the status of the pufferfish harvesting ban in Florida can be found through the Florida Fish and Wildlife Conservation Commission web site.

Mushroom toxins: Amanitin, Gyromitrin, Orellanine, Muscarine, Ibotenic Acid, Muscimol, Psilocybin, Coprine

1. Toxins

Mushroom poisoning is caused by consumption of raw or cooked fruiting bodies (mushrooms, toadstools) of a number of species of higher fungi. The term "toadstool" is commonly used for poisonous mushrooms. For individuals who are not trained experts in mushroom identification, there are, generally, no easily recognizable differences between poisonous and nonpoisonous species. Folklore notwithstanding, there is no reliable rule of thumb for distinguishing edible mushrooms from poisonous ones.

The toxins involved in mushroom poisoning are produced naturally, by the fungi themselves. Most mushrooms that cause human poisoning cannot be made nontoxic by cooking, canning, freezing, or any other means of processing. Thus, the only way to avoid poisoning is to avoid consumption of toxic species.

For Consumers: A Snapshot

Some wild mushrooms contain poisons that can cause illness, with symptoms ranging from mild to deadly. The poisons are *not* likely to be destroyed by washing, cooking, freezing, or canning. Many poisonous wild mushrooms are almost impossible to tell apart from those that aren't poisonous, and many cases of poisoning have happened in people who were using field guides and had a lot of experience, and were "sure" they had picked the right kind of mushroom. Likewise, folklore is *not* a reliable way to avoid poisonous mushrooms.

Some of the deadliest wild mushrooms don't cause obvious symptoms for hours or even days or weeks after they're eaten, and, by the time symptoms appear, it's likely that liver or kidney damage has already occurred. These kinds of cases often start out with symptoms that go away after a few hours and seem to be gone for 3 to 5 days, making the person think that he or she is better – but then much worse symptoms appear, often leading to death.

The best way to keep from getting sick from wild mushrooms is not to eat them. Some can make you sick even from eating a sauce that contains them, even if you don't eat the mushrooms themselves. It's much safer to get mushrooms from grocery stores that sell the products grown on professional mushroom farms.

2. Disease

Mushroom poisonings are generally acute, although onset of symptoms may be greatly delayed in some cases, and are manifested by a variety of symptoms and prognoses, depending on the amount and species consumed. The normal course of the disease varies with the dose and the mushroom species eaten. Each poisonous species contains one or more toxic compounds that are unique to few other species. Therefore, cases of mushroom poisonings generally do not resemble each other, unless they are caused by the same or very closely related mushroom species.

Almost all mushroom toxins may be grouped into one of the four categories outlined below. Because the chemistry of many mushroom toxins (especially the less deadly ones) is still unknown, and identification of mushrooms is often difficult or impossible, mushroom poisonings are generally categorized by their physiological effects. A broad overview of the four categories appears below, including a table that summarizes the onset time of symptoms after these poisons

are ingested, likely mushroom sources, and likely outcomes. This information is followed by a section containing more detailed descriptions, which includes a "miscellaneous" category. (Note: this information is not comprehensive; it is intended to provide only basic information, rather than to serve as a definitive diagnostic source.)

LIFE-THREATENING POISONS – protoplasmic poisons are known to kill several people each year in the United States

- ***protoplasmic poisons*** – life-threatening poisons that result in generalized destruction of cells, followed by organ failure. The protoplasmic poisons are the most likely to be fatal, due to irreversible organ damage. Victims who are hospitalized and given aggressive support therapy almost immediately after ingestion have a mortality rate of only 10%, whereas those admitted 60 or more hours after ingestion have a 50% to 90% mortality rate. However, some of the deadliest mushrooms do not result in symptoms until 6 to 72 hours after ingestion. Some result in symptoms that appear to resolve after a few hours, but, 3 to 5 days later, more serious symptoms begin that often end in death.

Life-Endangering Poisons – The following classes of poisons are generally not life-threatening, although death is possible in severe cases in which large amounts were consumed or the patient has additional health complications; e.g., organ transplant, hepatitis, HIV/AIDS, the elderly, etc. Observation of patients should continue and appropriate support therapy should be provided, as indicated.

- ***neurotoxins*** – compounds that cause neurological symptoms, such as profuse sweating, coma, convulsions, hallucinations, excitement, depression, spastic colon.

- ***gastrointestinal irritants*** – compounds that produce rapid, transient nausea, vomiting, abdominal cramping, and diarrhea.

- ***disulfiram-like toxins*** – mushrooms in this category generally are nontoxic and produce no symptoms, unless alcohol is consumed within 72 hours after eating them, in which case a short-lived, acute toxic syndrome is produced.

Table 1. Symptomatic diagnoses of mushroom poisonings

Onset Rapid (15 minutes to 2 hours after ingestion)		
Symptoms	**Cause**	**Prognosis**
Nausea and abdominal discomfort, sometimes with diarrhea and vomiting	Unknown toxins from numerous genera	Generally, rapid and complete recovery; serious cases may last 2 to 3 days and require fluid replacement
Profuse, prolonged sweating, tearing (lacrimation), salivation beginning 15-30 min after ingestion	Muscarine from *Clitocybe* or *Inocybe* spp.	Generally, complete recovery within approximately 2 h

Inebriation or hallucinations without drowsiness or sleep	Psilocybin from *Psilocybe, Paneolus, Gymnopilus, Conocybe,* or *Pluteus* spp.	Generally, complete and spontaneous recovery within 5-10 h; may take up to 24 h, with large doses
Delirium with sleepiness or coma developing within 1 or 2h after ingestion	Ibotenic acid/muscimol from *Amanita muscaria* or *A. pantherina*	Generally, alternating periods of drowsiness and excitement for several h, followed by total recovery
Onset Delayed (6 hours to 3 days after ingestion)		
Symptoms	**Cause**	**Prognosis**
Persistent and violent vomiting, abdominal pain, profuse, watery diarrhea beginning around 12 h after ingestion	alpha-, beta-, and gamma-amanitins from *Amanita phalloides* and its relatives; *Galerina autumnalis* and its relatives; or *Lepiota josserandii* and its relatives	Generally, apparent recovery a few hours after onset of symptoms, followed by a symptom-free period of 3 to 5 days, **which precedes a period of jaundice, loss of strength, coma, and, often, death**
Feeling of abdominal fullness and severe headache about 6 h after ingestion, vomiting, no diarrhea	Gyromitrin and related hydrazines from *Gyromitra esculenta* and its relatives	Generally, complete recovery within 2 to 6 days; may require correction of metabolic acidosis; **some deaths have occurred, due to liver failure**
Intense, burning thirst and frequent urination beginning 3-14 days after ingestion, followed by gastrointestinal disturbances, headache, pain in the limbs, spasms, and loss of consciousness	Orellanine from *Cortinarius orellanus*	Generally, recovery (including renal function) may require several months in less severe cases; **death from kidney failure may occur in severe cases**
Onset Conditional (on ingestion of alcohol within 72 hours)		
Symptoms	**Cause**	**Prognosis**
Flushing, palpitations, rapid heartbeat, rapid, labored breathing occur within 1/2 to 2 h after alcohol consumption, if alcohol was consumed within 72 h of mushroom ingestion	Coprine in *Coprinus atramentarius*	Generally, recovery is spontaneous and complete within a few to several hours after onset of symptoms

Some Specific Poisons, Sources, Symptoms, and Outcomes Within Each of the Four Major Toxin Categories

- ## Protoplasmic Poisons

Amatoxins: CDC/MMWR, Agricola

Several mushroom species, including the Death Cap or Destroying Angel (*Amanita phalloides*, *A. virosa*), the Fool's Mushroom (*A. verna*) and several of their relatives, along with the Autumn Skullcap (*Galerina autumnalis*) and some of its relatives, produce a family of cyclic octapeptides called amanitins.

Poisoning by the amanitins is characterized by a long latent period (range 6 to 48 hours, average 6 to 15 hours), during which the patient shows no symptoms. Symptoms appear at the end of the latent period in the form of sudden, severe seizures of abdominal pain, persistent vomiting and watery diarrhea, extreme thirst, and lack of urine production. If this early phase is survived, the patient may appear to recover for a short time, but this period generally will be followed by a rapid and severe loss of strength, prostration, and restlessness caused by pain.

Death occurs in 50% to 90% of the cases. The disease is progressive and causes irreversible liver, kidney, cardiac, and skeletal-muscle damage. Death may occur within 48 hours (large dose), but the disease more typically lasts 6 to 8 days in adults and 4 to 6 days in children. Two or three days after the onset of the later phase of the disease, jaundice, cyanosis, and coldness of the skin occur. Death usually follows a period of coma and, occasionally, convulsions. Autopsy usually reveals fatty degeneration and necrosis of the liver and kidney.

If recovery occurs, it generally requires at least a month and is accompanied by enlargement of the liver.

Hydrazines: Agricola, NIH/PubMed

Certain species of False Morel (*Gyromitra esculenta* and *G. gigas*) contain the protoplasmic poison gyromitrin, a volatile hydrazine derivative. Poisoning by this toxin superficially resembles *Amanita* poisoning, but is less severe.

There is generally a latent period of 6 to 10 hours after ingestion, during which no symptoms are evident, followed by sudden onset of abdominal discomfort (a feeling of fullness), severe headache, vomiting, and, sometimes, diarrhea. The toxin affects primarily the liver, but there are additional disturbances to blood cells and the central nervous system.

The mortality rate is relatively low (2% to 4%).

Poisonings with symptoms almost identical to those produced by *Gyromitra* also have been reported after ingestion of the Early False Morel (*Verpa bohemica*). The toxin is presumed to be related to gyromitrin, but has not yet been identified.

Orellanine: Agricola, NIH/PubMed

This type of protoplasmic poisoning is caused by the Sorrel Webcap mushroom (*Cortinarius orellanus*) and some of its relatives.

This mushroom produces orellanine, which causes a type of poisoning characterized by an extremely long asymptomatic latent period of 3 to 14 days. An intense, burning thirst (polydipsia) and excessive urination (polyuria) are the first symptoms. This may be followed by nausea, headache, muscular pains, chills, spasms, and loss of consciousness. In severe cases, severe renal tubular necrosis and kidney failure may result in death (15%) several weeks after the poisoning. Fatty degeneration of the liver and severe inflammatory changes in the intestine accompany the renal damage.

Recovery, in less severe cases, may require several months.

- ## Neurotoxins

Poisonings by mushrooms that cause neurological problems may be divided into three groups, based on the type of symptoms produced, and named for the substances responsible for these symptoms.

Muscarine Poisoning: CDC/MMWR, Agricola

Ingestion of any number of *Inocybe* or *Clitocybe* species (e.g., *Inocybe geophylla*, *Clitocybe dealbata*) results in an illness characterized primarily by profuse sweating. This effect is caused by the presence of high levels (3% to 4%) of muscarine. Muscarine poisoning is characterized by increased salivation, perspiration, and lacrimation (tearing) within 15 to 30 minutes after ingestion of the mushroom. With large doses, these symptoms may be followed by abdominal pain, severe nausea, diarrhea, blurred vision, and labored breathing. Intoxication generally subsides within 2 hours.

Deaths are rare, but may result from cardiac or respiratory failure, in severe cases.

Ibotenic Acid/Muscimol Poisoning: CDC/MMWR, NIH/PubMed, Agricola

The Fly Agaric (*Amanita muscaria*) and Panthercap (*Amanita pantherina*) mushrooms both produce ibotenic acid and muscimol. Both substances produce the same effects, but muscimol is approximately five times more potent than ibotenic acid.

Symptoms of poisoning generally occur within 1 to 2 hours after the mushrooms are ingested. Abdominal discomfort may be present or absent initially, but the chief symptoms are drowsiness and dizziness (sometimes accompanied by sleep), followed by a period of hyperactivity, excitability, derangement of the senses, manic behavior, and delirium. Periods of drowsiness may alternate with periods of excitement, but symptoms generally fade within a few hours.

Fatalities rarely occur in adults, but in children, accidentally consuming large quantities of these mushrooms may result in convulsions, coma, or other neurologic problems for up to 12 hours.

Psilocybin Poisoning: CDC/MMWR, NIH/PubMed, Agricola

A number of mushrooms belonging to the genera *Psilocybe, Panaeolus, Copelandia, Gymnopilus, Conocybe*, and *Pluteus* which, when ingested, produce a syndrome similar to alcohol intoxication (sometimes accompanied by hallucinations). Several of these mushrooms (e.g., *Psilocybe cubensis, P. mexicana, Conocybe cyanopus*) are eaten for their

psychotropic effects in religious ceremonies of certain native American tribes, a practice that dates to the pre-Columbian era.

The toxic effects are caused by psilocin and psilocybin. Onset of symptoms is usually rapid, and the effects generally subside within 2 hours. Poisonings by these mushrooms rarely are fatal in adults and may be distinguished from ibotenic acid poisoning by the absence of drowsiness or coma.

The most severe cases of psilocybin poisoning occur in small children, in whom large doses may cause hallucinations accompanied by fever, convulsions, coma, and death. These mushrooms are generally small, brown, nondescript, and not particularly fleshy; they are seldom mistaken for food fungi by innocent hunters of wild mushrooms.

Poisonings caused by intentional ingestion (other than that associated with religious tribal ceremonies) may involve overdoses or intoxications caused by a combination of the mushroom and some added psychotropic substance (such as PCP).

- **Gastrointestinal Irritants**

  Agricola

  Numerous mushrooms contain toxins that can cause gastrointestinal distress, including, but not limited to, nausea, vomiting, diarrhea, and abdominal cramps. In many ways, these symptoms are similar to those caused by the deadly protoplasmic poisons. The chief difference is that poisonings caused by these mushrooms (a list of names follows) have a rapid onset, rather than the delayed onset seen in protoplasmic poisonings. These mushrooms include the Green Gill (*Chlorophyllum molybdites*), Gray Pinkgill (*Entoloma lividum*), Tigertop (*Tricholoma pardinum*), Jack O'Lantern (*Omphalotus illudens*), Naked Brimcap (*Paxillus involutus*), Sickener (*Russula emetica*), Early False Morel (*Verpa bohemica*), Horse mushroom (*Agaricus arvensis*), and Pepper bolete (*Boletus piperatus*).

  The diarrhea and vomiting caused by some of these mushrooms (including the first five species mentioned above) may last for several days. Fatalities are relatively rare and are associated with dehydration and electrolyte imbalances caused by diarrhea and vomiting, especially in debilitated, very young, or very old patients. Replacement of fluids and other appropriate supportive therapy can prevent death in these cases.

  The chemistry of the toxins responsible for this type of poisoning is virtually unknown, but may be related to the presence, in some mushrooms, of unusual sugars, amino acids, peptides, resins, and other compounds.

- **Disulfiram-Like Poisoning**

  Agricola, NIH/PubMed

  The Inky Cap Mushroom (*Coprinus atramentarius*) is most commonly responsible for this poisoning, although a few other species also have been implicated. A complicating factor in this type of intoxication is that this species generally is considered edible, although consuming alcohol within 72 hours of eating it causes illness. The mushroom produces an unusual amino acid, coprine, which is converted to cyclopropanone hydrate in the human body. This compound interferes with the breakdown of alcohol. Consuming alcohol after

eating this mushroom causes headache, nausea and vomiting, flushing, and cardiovascular disturbances that last for 2 to 3 hours.

- **Miscellaneous Poisonings**

  Agricola, NIH/PubMed

  Young fruiting bodies of the sulfur shelf fungus *Laetiporus sulphureus* are considered edible. However, ingestion of this shelf fungus has caused digestive upset and other symptoms, in adults, and visual hallucinations and ataxia in a child.

3. Frequency

Accurate figures on the relative frequency of mushroom poisonings are difficult to obtain, and the fact that some cases are not reported must be taken into account. In California there were 6,317 reported mushroom poisoning cases between 1993 and 1997, resulting in 94 hospitalizations and one death (Nordt and Manoguerra, 2000). In Texas, in 2005 and 2006, there were 742 cases, resulting in 59 hospitalizations and no deaths (Barbee et al, 2009). Between 1959 and 2002, there were more than 28,000 reported mushroom poisonings, around the world, resulting in 133 deaths (Diaz, 2005a). Known cases are sporadic, and large outbreaks are rare. Poisonings tend to be grouped in the spring and fall, when most mushroom species are at the height of their fruiting stages.

While the actual incidence appears to be very low, the potential exists for grave problems. Poisonous mushrooms are not limited in distribution. Intoxications may occur at any time and place, with dangerous species occurring in habitats ranging from urban lawns to deep woods.

4. Sources

Cultivated commercial mushrooms of various species have not been implicated in poisoning outbreaks, although they may result in other problems, such as bacterial food poisoning associated with improper canning. Mushroom poisonings are almost always caused by ingestion of wild mushrooms that have been collected by nonspecialists (although specialists also have been poisoned). Most cases occur when toxic species are confused with edible species, and it is useful to ask victims or the people who provided the mushrooms what kind of mushrooms they thought they were picking. In the absence of a well-preserved specimen, the answer could narrow the suspects considerably. Intoxication also has occurred when people have relied on folk methods of distinguishing between poisonous and safe species.

Illnesses have occurred after ingestion of fresh, raw mushrooms; stir-fried mushrooms; home-canned mushrooms; mushrooms cooked in tomato sauce (which can render the sauce itself toxic, even when no mushrooms are consumed); and mushrooms that were blanched and frozen at home. Cases of poisoning by home-canned and frozen mushrooms are especially insidious, because a single incident may easily become a multiple outbreak when the preserved toadstools are carried to another location and consumed at another time.

Mistaken Identities

Specific cases of mistaken mushroom identity are frequent. For example, the Early False Morel *Gyromitra esculenta* (which is poisonous) is easily confused with the true Morel *Morchella esculenta* (which is not poisonous), and poisonings have occurred after consumption of fresh or

cooked *Gyromitra*. *Gyromitra* poisonings also have occurred after ingestion of commercially available "morels" contaminated with *G. esculenta*. The commercial sources for these fungi (which have not yet been successfully cultivated on a large scale) are field collection of wild morels by semiprofessionals.

Table 2 contains a short list of mushrooms often responsible for serious poisonings and the edible mushrooms with which they may be confused.

Table 2. Poisonous Mushrooms and Their Edible Look-Alikes

Mushrooms Containing Amatoxins		
Poisonous species	**Appearance**	**Mistaken for:**
Amanita tenuifolia (Slender Death Angel)	pure white	*Leucoagaricus naucina* (Smoothcap Parasol)
Amanita bisporigera (Death Angel)	pure white	*Amanita vaginata* (Grisette), *Leucoagaricus naucina* (Smoothcap Parasol), white *Agaricus* spp. (field mushrooms), *Tricholoma resplendens* (Shiny Cavalier)
Amanita verna (Fool's Mushroom)	pure white	*A. vaginata*, *L. naucina,* white *Agaricus* spp., *T. resplendens*
Amanita virosa (Destroying Angel)	pure white	*A. vaginata*, *L. naucina*, *Agaricus* spp., *T. resplendens*
Amanita phalloides (Deathcap)	pure white variety	*Amanita citrina* (False Deathcap), *A. vaginata, L. naucina, Agaricus* spp., *T. resplendens*
Buttons of *A. bisporigera, A. verna, A. virosa*	pure white	Buttons of white forms of *Agaricus* spp. Puffballs such as *Lycoperdon perlatum*, etc.
Amanita phalloides (Deathcap)	green = normal cap color	*Russula virescens* (Green Brittlegill), *Amanita calyptrodermia* (Hooded Grisette), *Amanita fulva* (Tawny Grisette), *Tricholoma flavovirens* (Cavalier Mushroom), *Tricholoma portentosum* (Sooty Head)
Amanita phalloides (Deathcap)	yellow variety	*Amanita caesarea* (Caesar's Mushroom)
Amanita brunnescens (Cleft Foot Deathcap)		*Amanita rubescens* (Blusher), *Amanita pantherina* (Panthercap)
Galerina autumnalis (Autumn Skullcap)	LBM (Little Brown Mushrooms)	"Little Brown Mushrooms," including *Gymnopilus spectabilis* (Big Laughing Mushroom) and other *Gymnopilus* spp., *Armillaria mellea* (Honey Mushroom)

Leucoagaricus brunnea (Browning Parasol)	LBM	*Lepiota* spp., *Leucoagaricus* spp., *Gymnopilus* spp. and other Parasol Mushrooms and LBMs
Lepiota josserandii, *L. helveola*, *L. subincarnata*	LBM	*Lepiota* spp., *Leucoagaricus* spp., *Gymnopilus* spp. and other Parasol Mushrooms and LBMs
Mushrooms that Produce Severe Gastroenteritis		
Chlorophyllum molybdites (Green Gill)		*Leucocoprinus rachodes* (Shaggy Parasol), *Leucocoprinus procera* (Parasol Mushroom)
Entoloma lividum (Gray Pinkgill)		*Tricholomopsis platyphylla* (Broadgill)
Tricholoma pardinum (Tigertop Mushroom)		*Tricholoma virgatum* (Silver Streaks), *Tricholoma myomyces* (Waxygill Cavalier)
Omphalotus olearius (Jack O'Lantern Mushroom)		*Cantharellus* spp. (Chanterelles)
Paxillus involutus (Naked Brimcap)		Distinctive, but when eaten raw or undercooked, will poison some people

- Also among the mushrooms that may be mistaken for edible species are those that produce mild gastroenteritis. They are too numerous to list here, but include members of many of the most abundant genera, including *Agaricus, Boletus, Lactarius, Russula, Tricholoma, Coprinus, Pluteus*, and others.

- The Inky Cap Mushroom (*Coprinus atramentarius*) is considered both edible and delicious. If alcohol is consumed within 72 hours of ingestion, the patient may suffer facial flushing, chest pain, nausea, and projectile vomiting, often mimicking an acute heart attack. Some other members of the genus *Coprinus* (Shaggy Mane, *C. comatus*; Glistening Inky Cap, *C. micaceus*; and others) and some of the larger members of the *Lepiota* family, such as the Parasol Mushroom.

- The potentially deadly Sorrel Webcap Mushroom (*Cortinarius orellanus*) is not easily distinguished from nonpoisonous webcaps belonging to the same distinctive genus, and all should be avoided. Other cases of mistaken identity may include ***psychotropic mushrooms*** (*Inocybe* spp., *Conocybe* spp., *Paneolus* spp., *Pluteus* spp.).

- Most of the psychotropic mushrooms are small, brown, and leathery (the so-called "Little Brown Mushrooms" or LBMs) in general appearance and relatively unattractive, from a culinary standpoint.

- The Sweat Mushroom (*Clitocybe dealbata*) and the Smoothcap Mushroom (*Psilocybe cubensis*) are small, white, and leathery. These small, unattractive mushrooms are

distinctive, fairly unappetizing, and not easily confused with the fleshier fungi normally considered edible. Intoxications associated with them are less likely to be accidental, although both *C. dealbata* and *Paneolus foenisicii* have been found growing in the same fairy ring area as the edible (and choice) Fairy Ring Mushroom (*Marasmius oreades*) and the Honey Mushroom (*Armillariella mellea*), and have been consumed when the picker has not carefully examined every mushroom picked from the ring.

- Psychotropic mushrooms more easily confused with edible mushrooms include the Showy Flamecap or Big Laughing Mushroom (*Gymnopilus spectabilis*), which has been mistaken for Chanterelles (*Cantharellus* spp.) and for *Gymnopilus ventricosus* found growing on wood of conifers in western North America.

- The Fly Agaric (*Amanita muscaria*) and Panthercap (*Amanita pantherina*) mushrooms are large, fleshy, and colorful. Yellowish cap colors on some varieties of the Fly Agaric and the Panthercap are similar to the edible Caesar's Mushroom (*Amanita caesarea*), which is considered a delicacy in Italy.

- Another edible yellow-capped mushroom occasionally confused with yellow *A. muscaria* and *A. pantherina* varieties is the Yellow Blusher (*Amanita flavorubens*). Orange to yellow-orange *A. muscaria* and *A. pantherina* may also be confused with the Blusher (*Amanita rubescens*) and the Honey Mushroom (*Armillariella mellea*).

- White to pale forms of *A. muscaria* may be confused with edible field mushrooms (*Agaricus* spp.).

- Young (button stage) specimens of *A. muscaria* also have been confused with puffballs.

5. Diagnosis

In the case of poisoning by the deadly Amanitas, important laboratory indicators of liver damage (elevated LDH, SGOT, and bilirubin levels) and kidney damage (elevated uric acid, creatinine, and BUN levels) will be present. Unfortunately, in the absence of dietary history, these signs could be mistaken for symptoms of liver or kidney impairment as the result of other causes (e.g., viral hepatitis). It is important that this distinction be made as quickly as possible, because the delayed onset of symptoms generally will mean that organ damage already has occurred.

A clinical testing procedure is currently available only for the most serious types of mushroom toxins, the amanitins. The commercially available method uses a 3H-radioimmunoassay (RIA) test kit and can detect sub-nanogram levels of toxin in urine and plasma. Unfortunately, it requires a 2-hour incubation period, and this is an excruciating delay in a type of poisoning that the clinician generally does not see until a day or two has passed. Amatoxins are eliminated in the urine, vomitus, and feces. They can be detected by chromatography, radioimmunoassay, and ELISA methods from bodily fluids and hepatorenal biopsies (Diaz 2005 b).

Since most clinical laboratories in this country do not use even the older RIA technique, diagnosis is based entirely on symptoms and recent dietary history. Despite the fact that cases of mushroom poisoning may be broken down into a relatively small number of categories based on symptomatology, positive botanical identification of the mushroom species consumed remains the only means of unequivocally determining the particular type of intoxication involved, and it is still vitally important to obtain such accurate identification as quickly as possible. Cases

involving ingestion of more than one toxic species, in which one set of symptoms masks or mimics another set, are among many reasons for needing this information.

Unfortunately, a number of factors (not discussed here) often make identification of the causative mushroom impossible. In such cases, diagnosis must be based on symptoms alone. To rule out other types of food poisoning and to conclude that the mushrooms eaten were the cause of the poisoning, it must be established that everyone who ate the suspect mushrooms became ill and that no one who did not eat the mushrooms became ill. Wild mushrooms, whether they were eaten raw, cooked, or processed, should always be regarded as prime suspects.

6. Target Populations

Poisonings in the U.S. occur when hunters of wild mushrooms (especially novices) misidentify and consume toxic species; when recent immigrants collect and consume poisonous American species that closely resemble edible wild mushrooms from their native lands; when mushrooms that contain psychoactive compounds are intentionally consumed by people who desire these effects; or by pre-school children who eat mushrooms they find growing in yards or gardens. In their analysis of mushroom exposures in California, Nordt and Manoguerra (2000) found that more than two-thirds of the reports were of children younger than 6 years old, but only 6% experienced any clinical effects.

All humans are susceptible to mushroom toxins. The poisonous species are ubiquitous, and geographical restrictions on types of poisoning that may occur in one location do not exist (except for some of the hallucinogenic LBMs, which occur primarily in the American Southwest and Southeast). Individual specimens of poisonous mushrooms also are characterized by individual variations in toxin content based on genetics, geographic location, and growing conditions. Intoxications may thus be more or less serious, depending not on the number of mushrooms consumed, but on the dose of toxin delivered.

In addition, although most cases of poisoning by higher plants occur in children, toxic mushrooms are consumed most often by adults. Occasional accidental mushroom poisonings of children and pets have been reported, but adults are more likely to actively search for, and consume, wild mushrooms for culinary purposes. Children are more seriously affected by the normally non-lethal toxins than are adults and are more likely to suffer very serious consequences from ingestion of relatively smaller doses. Adults who consume mushrooms are also more likely to recall what was eaten and when and are able to describe their symptoms more accurately than are children. Very old, very young, and debilitated persons of both sexes are more likely to become seriously ill from all types of mushroom poisoning; even from types generally considered to be mild.

Many idiosyncratic adverse reactions to mushrooms have been reported. Some mushrooms cause certain people to become violently ill, while not affecting others who consumed part of the same mushroom cap. Factors such as age, sex, and general health of the consumer do not seem to be reliable predictors of these reactions, and they have been attributed to allergic or hypersensitivity reactions and to inherited inability of the victim to metabolize certain unusual fungal constituents (such as the uncommon sugar trehalose). These reactions probably are not true poisonings, as the general population does not seem to be affected.

7. Food Analysis

The mushroom toxins can, with difficulty, be recovered from poisonous fungi, cooking water, stomach contents, serum, and urine. Procedures for extraction and quantitation are generally elaborate and time-consuming, and, in most cases, the patient will have recovered by the time an analysis is made on the basis of toxin chemistry. The exact chemical natures of most of the toxins that produce milder symptoms are unknown.

Chromatographic techniques (TLC, GLC, HPLC) exist for the amanitins, orellanine, muscimol/ibotenic acid, psilocybin, muscarine, and the gyromitrins. The amanitins may also be determined by commercially available 3H-RIA kits or ELISA test kits.

The most reliable means of diagnosing a mushroom poisoning remains botanical identification of the fungus that was eaten. Correctly identifying the mushrooms before they are eaten will prevent accidental poisonings. Accurate post-ingestion analyses for specific toxins, when no botanical identification is possible, may be essential only in cases of suspected poisoning by the deadly *Amanitas*, since prompt and aggressive therapy (including lavage, activated charcoal, and plasmapheresis) can greatly reduce the mortality rate.

8. Examples of Outbreaks

For more information about recent outbreaks, see the Centers for Disease Control and Prevention's Morbidity and Mortality Weekly Reports.

9. Other Resources

- Loci index for genomes *A. arvensis* | *L. sulphureus* | *V. bohemica* | *G. esculenta* | *I. geophylla* | *C. dealbata* | *A. muscaria* | *A. pantherina* | *Psilocybe spp.* | *C. rickenii* | *P. acuminatus* | *Pluteus spp.* | *C. molybdites* | *T. pardinum* | *O. illudens* | *P. involutus* | *A. virosa* | *Cortinarius spp.* | *C. atramentarius*

- GenBank Taxonomy database

10. Molecular Structures

Amanitin

Orellanine

Muscarine

Ibotenic acid

Muscimol

Psilocybin

Gyromitrin

Coprine

Additional reading

Barbee G, Berry-Cabán C, Barry J, Borys D, Ward J, Salyer S. Analysis of mushroom exposures in Texas requiring hospitalization, 2005-2006, J Med Toxicol. 2009 Jun;5(2):59-62.

Diaz JH. Evolving global epidemiology, syndromic classification, general management, and prevention of unknown mushroom poisonings, (2005a) Crit Care Med 33(2)419-426.

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Nordt SP and Manoguerra A. 5-Year analysis of mushroom exposures in California, West J Med. 2000 November; 173(5): 314–317.

Bad Bug Book
Foodborne Pathogenic Microorganisms and Natural Toxins

Aflatoxins

1. Toxin

The aflatoxins (AFs) are mycotoxins produced by certain fungi and can cause serious illness in animals and humans. The four major aflatoxins are AFB_1, AFB_2, AFG_1, and AFG_2. In adverse weather or under poor storage conditions, these toxins are produced mainly by certain strains of *Aspergillus flavus* and *A. parasiticus* in a broad range of agricultural commodities, such as corn and nuts.

The name "aflatoxin" reflects the fact that this compound was first recognized in damaged peanuts contaminated with *Aspergillus flavus*. The aflatoxins then were described according to other mechanisms (i.e., on the basis of their blue or green fluorescence under UV light and relative chromatographic mobility after thin-layer chromatographic separation).

Another aflatoxin, aflatoxin M_1 (AFM_1), is produced by mammals after consumption of feed (or food) contaminated by AFB_1. Cows are able to convert AFB_1 into AFM_1 and transmit it through their milk. Although AFM_1 in milk is, by far, not as hazardous as the parent compound, a limit of 0.5 parts per billion is applied, largely because milk tends to constitute a large part of the diet of infants and children.

For Consumers: A Snapshot

Aflatoxins are toxic substances produced by some kinds of fungus that can grow on food. People who eat food that contains high levels of aflatoxins can become sick. To date, there has never been a human illness outbreak caused by aflatoxins in the U.S., where foods are carefully regulated and inspected to prevent such an occurrence, but some developing countries have had outbreaks. One of the aflatoxins is among the strongest known carcinogens (substances that cause cancer). Scientists have pinpointed a site where this aflatoxin appears to cause a mutation in human DNA. Aflatoxins can lead to liver and immune-system problems. The combination of hepatitis B infection and eating foods contaminated with aflatoxin appears to make the risk of liver cancer especially high. Foods in which aflatoxins commonly are found (unless regulations and inspections prevent it, as in the U.S.) include corn, sorghum, rice, cottonseed, peanuts, tree nuts, dried coconut meat, cocoa beans, figs, ginger, and nutmeg.

Aflatoxins can cause illness in animals, and contaminated pet foods caused outbreaks and deaths among U.S. dogs and cats in 1998 and 2005. Cows are able to metabolize – process – aflatoxin. The substance (metabolite) that results after the cow processes the aflatoxin then may appear in the cow's milk, but is less toxic than the aflatoxin itself. Milk is routinely tested for this substance. In some developing countries, this metabolite also is found in the breast milk of human mothers who eat aflatoxin-contaminated foods.

In the United States, strict regulations in place since 1971, as well as FDA monitoring of the food supply and the population's consumption of a diverse diet, have prevented human health problems. (See FDA guidelines.) At the time of this writing, no outbreaks of aflatoxicosis – disease caused by aflatoxins – have been reported in humans in the U.S. Acute toxicosis has occurred in domestic animals, but this is rare. However, aflatoxin-induced chronic and acute disease is common in children and adults in some developing countries.

2. Disease

Chronic exposure to aflatoxin well above the FDA guideline affects many organs; however, the major target is the liver. AFs are hepatotoxic in humans and animals. Food-related exposures to AFs and the resulting aflatoxicosis can range from acute to chronic, and illness can range from mild to severe, including development of cirrhosis (severe liver damage) and may result in development of liver cancer. AFB_1 is the most potent known natural carcinogen.

It is difficult to prove that a disease is caused by AFs. It is possible to test tumor tissue for biomarkers or characteristic genetic damage. Even in cases where AF exposures have been of long duration and are well above the U.S. limits, it is unlikely that they are the only agents responsible for the outcome. However, there is reliable evidence, from animal studies and case reports and long-term studies of human health outcomes, that AFs pose an important danger to human and animal health unless properly regulated.

- **Mortality**: Documented epidemics of AF poisoning in the following countries illustrate mortality rates from outbreaks:

    - In northwest India, in 1974, there were 108 fatalities from 397 illnesses. AF levels of 0.25 to 15 mg/kg were found in corn.

    - In 1982, in Kenya, there were 20 hospital admissions, with a 60% mortality rate, with AF intake at 38 µg/kg of body weight.

    - In 1988, in Malaysia, 13 Chinese children died of acute hepatic encephalopathy after eating Chinese noodles. Aflatoxins were confirmed in postmortem samples from the patients.

    - In 2004 and 2005, one of the largest aflatoxicosis outbreaks on record occurred in rural Kenya, resulting in illness in 317 people, 125 of whom died. AF-contaminated homegrown maize with an average concentration of 354 ng/g was the source of the outbreak.

- **Toxic dose**: The toxic level of AF in humans is largely unknown. In one example, a laboratory worker who intentionally ingested AFB_1 at 12 µg/kg body weight for 2 days developed a rash, nausea, and headache, but recovered without ill effect. In a 14-year follow-up of the worker, a physical examination and blood chemistry, including tests for liver function, were normal.

  See the "Mortality" section, above, for examples of concentrations of AF in various foods that have caused illness and death in humans.

  In animals, the effects of AFs on health depend on the species of the animal, level and duration of exposure, and nutritional status. Among various animals, median lethal dose (i.e., LD_{50}) values obtained with single doses showed wide variation, ranging from 0.3 mg/kg body weight in rabbits to 18 mg/kg body weight in rats.

  AFs have been found to be moderately to highly toxic and carcinogenic in almost every animal species tested, including monkeys, although AFs do not affect all animals equally. The main factor in tolerance relates to the nature of the digestive system. Ruminants are more tolerant, and swine, chickens, ducks, and ducklings (and pet and wild birds) are more sensitive. Other factors contributing to differences in animal susceptibility to AFs include breed variety, nutrition, sex, age, environmental stress, and presence of other

disease agents. However, carcinogenicity in production livestock, resulting from the consumption of AF-contaminated feed, is seldom seen.

- **Onset**: Not applicable.
- **Illness / complications:**

  From acute exposure: Acute exposure to high doses of AFs can result in aflatoxicosis, with the target organ being the liver, leading to serious liver damage. AFs inhibit the normal functions of the liver, including carbohydrate and lipid metabolism and protein synthesis.

  From chronic exposure at sublethal doses: cancer, impaired protein formation, impaired blood coagulation, toxic hepatitis, and probable immunosuppression. In animals, AFs may cause, in addition, reduced weight gain and reduced feed-conversion efficiency.

  AFB_1 is the most potent known natural carcinogen and is the most abundant of the AFs. The International Agency for Research on Cancer has classified AFB_1 as a group 1 carcinogen and AFM_1 as a group 2b carcinogen (carcinogenic to laboratory animals and possibly carcinogenic to humans, respectively). Combined exposure to aflatoxin and hepatitis B increases the risk for development of human hepatocellular carcinoma (HCC).

  As noted, the diagnosis of chronic aflatoxicosis is difficult without sophisticated laboratory facilities.

  Other significant health effects of AF exposure follow from the finding that they are probably immunosuppressive in humans. AFs have been shown primarily to affect the cellular immune processes in most of the laboratory animal species studied. Some animals exhibit a decrease in antibody formation, and there is evidence of transplacental movement of AFs, allowing embryonic exposure and reducing immune responses in offspring.

  Symptoms: The disruption and inhibition of carbohydrate and lipid metabolism and protein synthesis associated with aflatoxicosis can lead to hemorrhaging, jaundice, premature cell death, and tissue necrosis in liver and, possibly, other organs. Other general symptoms include edema of the lower extremities, abdominal pain, and vomiting.

- **Duration of symptoms**: Poorly described in the literature.
- **Route of entry**: Oral.
- **Pathway**: There is sufficient evidence that AFB_1 can interact with DNA, producing damage. If the DNA is not repaired, a mutation can occur that may initiate the cascade of events required to produce cancer. This has been partly elucidated, as follows.

  After activation by cytochrome P450 monooxygenases, AFB_1 is metabolized to form a highly reactive metabolite, AFB_1-exo-8,9-epoxide. The exo-epoxide binds to the guanine moiety of DNA at the N7 position, forming trans-8,9-dihydro-8-(N7-guanyl)-9-hydoxyAFB_1 adducts, which can rearrange and form a stable adduct. This can be measured in tumor tissues. AFB_1-DNA adducts can result in GC-to-AT transversions. This specific mutation at codon 249 of the p53 tumor suppressor gene may be important in the development of HCC. Studies of liver-cancer patients in Southeast Asia and sub-Saharan Africa, where AF contamination in foods was high, have shown that a mutation in the p53 at codon 249 is associated with a G-to-T transversion.

  Biomarkers continue to serve as important tools in the epidemiology of HCC.

3. Frequency

In 2004, according to the Worldwide Regulations for Mycotoxins 2003, a Compendium published by the Food and Agriculture Organization, more than 76 countries have legislated limits on aflatoxins, ranging from 0 to 35 ng/g. Subsequently, in developed countries, AF contamination has rarely occurred in foods at levels that cause acute aflatoxicosis in humans.

AF acute and chronic exposures are more likely to occur in developing countries where no regulatory limits, poor agricultural practices in food handling and storage, malnutrition, and disease are problems. Aflatoxicosis in humans has been reported in many countries, including India, China, Thailand, Ghana, Kenya, Nigeria, Sierra Leone, and Sudan. Human epidemiologic studies were initiated, in 1966, in Africa.

To date, in the U.S., no human aflatoxicosis outbreak has been reported; however, dogs died in an outbreak, in 1998. In 2005, a number of dogs and cats died from eating aflatoxin-contaminated pet food.

4. Sources

In the U.S., AFs are commonly found in corn (maize), sorghum, rice, cottonseed, peanuts, tree nuts, copra, cocoa beans, figs, ginger, and nutmeg. AFM_1 may be found in milk and dairy products. Aflatoxin M_1 also may be found in human breast milk, as has been the case in Ghana, Kenya, Nigeria, Sudan, Thailand, and other countries, from a mother's chronic exposure to dietary AFs.

5. Diagnosis

People who have aflatoxicosis might exhibit the following characteristics.

- Liver damage may be evidenced by jaundice and its characteristic yellowing of tissues.
- Gall bladder may become swollen.
- Immunosuppression may provide an opportunity for secondary infections.
- Vitamin K functions may decrease.
- High levels of AFB_1-albumin adducts may be present in plasma.

AF exposure can be monitored through the use of biomarkers that detect the presence of AF metabolites in blood, milk, and urine, and excreted DNA adducts and blood-protein adducts. AFB_1-albumin adducts can be measured in blood; AFM_1 and AFB_1-DNA adduct (AFB_1-guanine adduct) can be detected in the urine of people consuming sufficient amounts of AFB_1.

6. Target Populations

Human susceptibility to AFs can vary with sex, age, health, nutrition, environmental stress, and level and duration of exposure. In many cases, exposure is due to consumption of a single, affected dietary staple. Also see "Frequency" section, above.

7. Food Analysis

Since 1963, considerable effort has been focused on development and refinement of procedures for sampling, sample preparation, extraction, purification, isolation, separation, and quantitation of AFs in foods, with sampling being the most difficult step in mycotoxin determination.

It is known that AFs are heterogeneously distributed in agricultural commodities. There have been reports of AF concentrations in excess of 1,000,000 ng/g for individual peanut kernels; 5,000,000 ng/g for cottonseed; and more than 4,000,000 ng/g in corn kernels. Therefore, the sampling variability encountered at this step is the largest in the total testing procedure.

Two important aspects that can affect sampling variability include the sample-selection procedure and the distribution among contaminated particles within a lot. Using proper sampling equipment and procedures can reduce the effects of sample selection. Increasing sample size can reduce the effects of the distribution of contaminated particles within a lot.

A bulk sample must be taken following a sampling plan, so that it is accurately representative of the toxin levels present throughout the lot. A subsample is removed from the bulk sample and subjected to sample preparation. The subsample is comminuted with proper grinding and mixing mills. The sample preparation variability decreases with decreasing particle size. A test sample is removed from the properly comminuted sample for analysis.

Analytical methods can be divided into quantitative or semiquantitative assays and rapid screening tests. Sample cleanup is a time-consuming step and usually consists of extraction with solvent, liquid-liquid partition, and/or chromatographic separation and determination. Thin-layer chromatography (TLC) is among the most widely-used analytical methods. This simple and inexpensive technique is especially useful for AF analysis in developing countries, screening purposes, and multi-mycotoxin analysis.

Since the late 1970s, AF-specific antibodies have been developed. The antibody development has led to the development of enzyme-linked immunosorbent assays (ELISAs) for AFs. The ELISAs are mainly used in screening methods.

With advances in instrumentation, chromatographic methods for AFs have expanded from TLC to high-performance liquid chromatography (LC) with fluorescence detection. Hyphenated methods, such as LC/mass spectrometry (MS) or LC/MS-MS, have also been developed for AF quantitation and confirmation of identities.

Emerging analytical technologies for AF include solid-phase micro-extraction, surface-plasmon resonance, fiber-optic sensors, electrochemical immunosensors, fluorescence-based immunoassays, and the use of molecularly imprinted polymers for binding the AFs. Recently, non-invasive analyses, such as near-infrared spectrometry, have been used, with limited success, for detecting the occurrence of *A. flavus*-infected corn kernels and correlating these occurrences with AF levels.

All AF methods that were internationally validated by collaborative studies are described in Chapter 49 of the AOAC Official Methods of Analysis, 18th edition.

8. Examples of Outbreaks

- For more information on outbreaks see the Centers for Disease Control and Prevention's Morbidity and Mortality Weekly Reports.

9. Other Resources:

- NIH/PubMed: Aflatoxins – Provides a list of research abstracts contained in the National Library of Medicine's MEDLINE database for this organism or toxin.
- Agricola: Aflatoxins – Provides a list of research abstracts contained in the National Agricultural Library database for this organism or toxin.

- Loci index for genomes Aspergillus flavus | Aspergillus parasiticus
- Available from the GenBank Taxonomy database, which contains the names of all organisms that are represented in the genetic databases with at least one nucleotide or protein sequence.

10. Molecular Structural Data:

Aflatoxins B1, B2, G1, G2, and M1

Gempylotoxin

For Consumers: A Snapshot

The fish escolar and its relative oilfish contain an oil that includes a waxy substance humans can't digest. In some people, eating even small amounts of these fish can cause oily diarrhea (orange or brownish-green), abdominal cramps, nausea, vomiting, and headache. Usually, not much fluid is lost from the body with the diarrhea caused by these fish, and the symptoms generally go away in a day or two. Some people don't get sick if they eat small amounts of these fish; they enjoy them and continue to eat them. But these fish are called different names in different areas. If packages were to use those names on the label, the people who bought them might not know that they're really getting escolar or oilfish and that it could make them sick. For example, oilfish are sometimes called "cocco." Other common names for escolar are butterfish, white tuna, and walu. The FDA does not allow these fish to be imported or sold across state lines using these different names. To help protect yourself, buy your fish from a reputable market, to help ensure that the fish in the package really is what the label says it is. The box called "DNA Barcoding," below, is about a new method the FDA is using to tell what kind of fish is in a package.

1. Toxin

Gempylotoxin is an indigestible wax, composed of C32, C34, C36, and C38 fatty acid esters, with the main component C34H66O2 (Ukishima, *et al*.), generally found in the fish escolar (*Lepidocybium flavobrunneum*) and its relative oilfish (*Ruvettus pretiosus*), sometimes called cocco.

Some consumers continue to eat these fish, despite the fact that they may have a purgative effect. This may be due to personal preference, or consumers may unwittingly eat these fish if the product is not identified as escolar or oilfish and is instead marketed under different names. For additional information on vernacular or misleading names used for these species, see the Sources section, below. Photos of the fish and packaging also appear in the Sources section.

FDA advises against the sale of these fish in intrastate / interstate commerce, and requests that seafood manufacturers / processors inform potential buyers / sellers, etc., of the purgative effect associated with consumption of these fish. FDA district offices have been asked to refer any consumer complaints or questions associated with consumption of these fish to the FDA Center for Food Safety and Applied Nutrition. Questions regarding escolar and relative species may be directed to the Division of Seafood Safety, Office of Food Safety, CFSAN, 301-436-2300. *(Based on Health Hazard Evaluation No. 2841, Health Hazard Evaluation Board, CFSAN, FDA, 1992.)*

In an analysis by Japanese researchers, escolar's muscle contained about 20% lipid, and 88.8% consisted of wax. The wax was composed of C32, C34, C36 and C38 compounds, and the main component was $C_{34}H_{66}O_2$. The alcohol components were mainly C16:0 and C18:1, as well as those of sperm whale (*Physeter catodon*) wax. The fatty acid components were mainly C18:1 and smaller amounts of highly unsaturated fatty acids. See also the FDA Fish and Fishery Hazards and Controls Guidance, Fourth Edition, chapter 6.

2. Disease

Humans can't digest this wax, which, in some people, acts as a purgative if consumed. The resulting illness is called gempylid fish poisoning or gempylotoxism.

- **Mortality**: None known.
- **Onset**: Symptoms have been reported to start between 1 and 90 hours after the fish is consumed, with a median onset of 2.5 hours.
- **Symptoms**: Diarrhea, often consisting of an oily orange or brownish-green discharge (keriorrhoea), without major fluid loss; abdominal cramps; nausea; headache; and vomiting.
- **Duration**: Symptoms usually abate within 1 to 2 days.
- **Route of entry**: Oral.

3. Frequency

Cases may occur sporadically (i.e., in isolation from one another) or in clusters, usually when the fish is eaten in group settings. (See "Examples of Outbreaks" section, below.)

4. Sources

Symptoms usually are associated with ingestion of escolar (*Lepidocybium flavobrunneum*) or oilfish (*Ruvettus pretiosus*). Other products have been implicated in illness (including butterfish, rudderfish, walu, white tuna, and Taiwanese seabass). In most cases, these products were actually escolar or oilfish, but were marketed under inappropriate local or vernacular names, such as those used where the species was harvested (e.g. walu, butterfish). Species substitution or misbranding occurs when a deceptive and misleading name is used (e.g., white tuna or Taiwanese seabass). The FDA maintains a guide to acceptable market names for food fish sold in interstate commerce (The Seafood List), to avoid this confusion among consumers and resulting inadvertent illness.

Additional deep-sea fish species, such as orange roughy (*Hoplostethus atlanticus*) and oreo dory (*Allocyttus* spp., *Pseudocyttus* spp., *Oreosoma* spp., and *Neocyttus* spp.), are known to contain lesser amounts of the same indigestible wax esters. Sensitive people also may experience symptoms from consumption of these fish.

Improperly handled escolar and oilfish also have been associated with scombrotoxin (histamine) poisoning, the topic of a separate chapter of the Bad Bug Book.

Images and other information from the Regulatory Fish Encyclopedia:

Escolar

Oilfish

Photos of Commercial Product and Packaging

Top photos by:
Whole escolar: Warren Savary, FDA/ORA
Escolar fillet: Warren Savary, FDA/ORA
Whole oilfish: D. Mellen, FDA/ORA
Oilfish fillet : D. Mellen, FDA/ORA

Bottom photos by:
Fillet: Dianne Millazo, FDA, Richmond, VA RP
Label: Amber Chung, FDA, NOVA RP

5. Diagnosis

Diagnosis is per symptoms, particularly of oily, orange or greenish-brown diarrhea, and history of having consumed this type of fish.

6. Target Populations

Not everyone who eats the fish becomes ill to the same extent. Level of illness may be related to the quantity eaten.

7. Food Analysis

The following articles provide information relevant to food analysis of the oils containing high levels of indigestible wax esters in these fish, as well as methods for identification of those species.

- Review Article on Fish-induced Keriorrhea: Ling KH, Nichols PD, But PPH. (2009). Fish-induced Keriorrhea. In: Taylor, S. L. (Ed.), *Advances in Food and Nutrition Research*, **57**: 1–52. Academic Press, San Diego.

- Ling KH, Cheung CW, Cheng SW, Cheng L, Li S-L, Nichols PD, Ward RD, Graham A, But PPH. Rapid detection of oilfish and escolar in fish steaks: A tool to prevent keriorrhea episodes. *Food Chemistry*, 110 (2008), 538-546.

- Nichols PD, Mooney BD, Elliot NG. Unusually high levels of non-saponifiable lipids in the fishes escolar and rudderfish: Identification by gas and thin-layer chromatography. *Journal of Chromatography A*, 936 (2001) 183-191 [CSIRO Marine Research, GPO Box 1538, Hobart, Tasmania 7000, Australia. peter.nichols@marine.csiro.au] | PubMed.

- Berman P, Harley EH, Spark AA. Keriorrhoea - the passage of oil per rectum - after ingestion of marine wax esters. *S. Afr. Med. J.* May 23, 1981;59(22), 791-2 | PubMed

- Halstead BW. Poisonous and Venomous Marine Animals of the World, Vol. II, U.S. Government Printing Office, Washington, DC, 1967

- Nevenzel JC, Rodegker W, Mead JF The lipids of Ruvettus pretiosus muscle and liver. *Biochemistry*. 1965 Aug;4(8):1589-94 | PubMed

- Ukishima Y, Masui T, Matsubara S, Goto R, Okada S, Tsuji K, Kosuge T. Wax components of escolar (*Lepidocybium flavobrunneum*) and its application to base of medicine and cosmetics. *Yakugaku Zasshi*. Nov 1987;107(11):883-90 [Article in Japanese] | PubMed

8. Examples of Outbreaks

An outbreak that occurred in New South Wales, in October 2001, provides an example. Of 44 people who attended a conference at which lunch was served, 22 became ill, with a median post-lunch incubation period of 2.5 hours. Among those, all of the 17 who met the case definition had eaten fish for lunch; none of the attendees who did not become ill had eaten fish. Eighty percent of the people who became ill had diarrhea, often oily; half had abdominal cramps and almost half had nausea; more than one-third had a headache; and one-quarter had vomiting. Analysis of the oil in the fish that had been served for lunch was consistent with escolar.

9. Other Resources

CDC/MMWR: Gempylotoxin: CDC's Morbidity and Mortality Weekly Report.

At the time of this writing, a search of the term "gempylid" resulted in no current reports of gempylid fish poisoning in CDC's MMWR. However, if such reports should emerge, they would appear at the above link, which readers may check periodically.

NIH/PubMed: Gempylotoxin: Research abstracts in the National Library of Medicine's MEDLINE database.

Agricola: Gempylotoxin: Research abstracts in the USDA NAL database. At the time of this writing, a search of the term "gempylid" resulted in no current reports of gempylid fish poisoning in NAL's Agricola. However, if such reports should emerge, they would appear at the above link, which readers may check periodically.

DNA Barcoding

After a fish is turned into fillets or steaks, it can be very hard to determine exactly what species it is. FDA scientists are now using DNA barcoding to find out. DNA barcoding uses genetic material in fish to identify them. This method of definitive identification helps the FDA enforce policies on proper labeling of escolar and other fish.

A different fish described in another chapter of this book (pufferfish, in the tetrodotoxin chapter) provides another example of DNA barcoding's utility. Pufferfish can be poisonous, depending on the type of pufferfish and the parts that are eaten. Some kinds are considered a delicacy, sold in specialty markets, after highly trained cutters have removed the poisonous parts. FDA allows only one type of pufferfish, pre-cut, to be imported into the U.S. Some importers have tried to bring pufferfish into the U.S. labeled as something else, to avoid these strict limits. DNA barcoding is another tool the FDA can use to ensure that the labels on shipments are accurate, to protect the public's health.

Pyrrolizidine Alkaloids

For Consumers: A Snapshot

Poisoning by these toxins, which are found in some plants, is rare in the U.S. – but when it does happen, it can be serious and can lead to death, usually from liver damage. One of them is now recognized as a potential cause of cancer. Most of the known poisoning cases have been linked to dietary supplements, such as herbal remedies or teas made from plants (comfrey, for example) that have been reported to contain the toxins.

Although the poison usually is out of the body within a day, the symptoms of the poisoning might not appear for days or weeks. By the time they seek medical attention, patients often have forgotten what they ate or drank, so diagnosing this illness can be hard. The symptoms that sometimes lead people to get help may include pain, particularly in the right upper part of the abdomen; nausea; vomiting; swollen belly; swollen veins on the belly; puffiness from fluid; and fever. The skin and whites of the eyes may turn yellow. Whether or not people recover from the liver damage these toxins cause depends partly on how much they took and for how long. In some cases, if the dose was low or short-term, the liver can heal itself. In severe cases, it can't, and without a liver transplant, the person may die. The lungs also may be damaged in severe cases, and this also may lead to death.

Medical care is aimed at treating the symptoms; for example, relieving the dangerous fluid build-up that can occur with liver damage.

1. Toxin

Pyrrolizidine alkaloids are a large class of naturally occurring alkaloids containing pyrrolizidine rings. More than 600 pyrrolizidine alkaloids are known. They are widely distributed in the plant kingdom, particularly in the Boraginaceae, Compositae, and Leguminosae families. Some of these alkaloids cause illness in humans and other animals.

2. Disease

- **Mortality:** Possible, when liver or lung damage is extensive.
- **Toxicity dose:** Variable among different pyrrolizidine alkaloids.
- **Onset:** Evidence of toxicity may not become apparent for days or weeks after the alkaloid is ingested.
- **Illness / complications:** Most cases of pyrrolizidine alkaloid toxicity result in moderate to severe liver damage. In some cases, the lungs are affected; pulmonary edema and pleural effusions have been observed. Lung damage may be prominent and has been fatal. Chronic illness from ingestion of small amounts of the alkaloids over a long period proceeds through fibrosis of the liver to cirrhosis. The carcinogenic potential of some pyrrolizidine alkaloids has been proven in rodents, and the National Toxicology Program recently has accepted riddelliine as a human carcinogen.

Treatment is symptomatic. Liver transplantation may be needed in severe cases.

- **Symptoms:** Gastrointestinal symptoms usually are the first sign of intoxication. They consist predominantly of abdominal pain, with vomiting, and development of ascites. Other early clinical signs include nausea and acute upper gastric pain, acute abdominal distension with prominent dilated veins on the abdominal wall, fever, and biochemical evidence of liver dysfunction. Jaundice may be present.

- **Duration:** Death may ensue from 2 weeks to more than 2 years after poisoning, but patients may recover almost completely if the alkaloid intake is discontinued and the liver damage has not been too severe.

- **Route of entry:** Oral.

- **Pathway:** Mediated by cytochrome P450.

3. Frequency

Worldwide, reports of pyrrolizidine alkaloid intoxication are associated mainly with consumption of dietary supplements containing pyrrolizidine alkaloids and grains contaminated with weeds that contain pyrrolizidine alkaloids. Although the occurrence has been rare, there have been periodic reports of pyrrolizidine alkaloid intoxication in the United States, mainly due to consumption of herbal teas and dietary supplements that contained pyrrolizidine alkaloids; mainly the herb comfrey (*Symphytum* spp.).

4. Source

The plants most frequently implicated in pyrrolizidine poisoning are members of the Boraginaceae, Compositae (also called Asteraceae), and Leguminosae (also called Fabaceae) families. Pyrrolizidine alkaloid intoxication is caused by consumption of plant material containing these alkaloids. The plants may be consumed as food, for medicinal purposes, or as contaminants of other agricultural crops. Cereal and forage crops are sometimes contaminated with pyrrolizidine-producing weeds, and the alkaloids may thus contaminate flour and other foods, including milk from cows feeding on these plants and honey from bees foraging on plants containing pyrrolizidine alkaloids.

5. Diagnosis

Diagnosis of poisoning from pyrrolizidine alkaloids often is difficult, since they usually are excreted within 24 hours, while symptoms of the poisoning might not appear until days or weeks after the toxins were ingested. Key clinical features of the veno-occlusive disease that typically is indicative of pyrrolizidine alkaloids may include hyperbilirubinemia, painful hepatomegaly, and fluid retention. Diagnosis usually is made on the basis of symptoms and on patients' reports of having ingested substances associated with pyrrolizidine alkaloids.

6. Target Populations

All humans are believed to be susceptible to the hepatotoxic pyrrolizidine alkaloids. Males are more susceptible than females, and fetuses and children show the highest sensitivity. Home remedies and consumption of herbal teas in large quantities can be a risk factor and are the most likely causes of alkaloid poisonings in the U.S. In 2001, FDA advised all dietary supplement manufacturers to remove from the market products that contained comfrey and were intended for internal use.

7. Food Analysis

The pyrrolizidine alkaloids can be isolated from the suspect commodity by any of several standard alkaloid extraction procedures. The toxins are identified by thin-layer chromatography. The pyrrolizidine ring is first oxidized to a pyrrole, followed by spraying with Ehrlich reagent, which gives a characteristic purple spot. A colorimetric test employing Ehrich reagent also can be used to detect most common pyrrolizidine alkaloids, except the otonecine-type. Liquid and gas-liquid chromatography, in conjunction with mass spectrometric methods, also are available for identifying the alkaloids in trace amounts.

8. Examples of outbreaks

Intoxication reported from Afghanistan's Gulran province in 2008.

List of Morbidity and Mortality Weekly Reports, from the Centers for Disease Control and Prevention, relating to this toxin.

List of research abstracts from the National Library of Medicine's MEDLINE database.

List of research abstracts from the National Agricultural Library database.

9. Resources

- TOXNET
- FDA Advises Dietary Supplement Manufacturers to Remove Comfrey Products From the Market
- Prakash AS *et al*. Pyrrolizidine alkaloids in human diet. Mutation Research 1999, 443: 53-67.
- Fu PP *et al*. Pyrrolizidine alkaloids--genotoxicity, metabolism enzymes, metabolic activation, and mechanisms. Drug Metabolism Reviews, 2004, 36(1):1-55.
- Wiedenfeld H, Edgar J. Toxicity of pyrrolizidine alkaloids to humans and ruminants. Phytochemical Reviews 2011, 10:137–151.

10. Molecular Structures

Pyrrolizidine alkaloids of *Symphytum* spp.

Pyrrolizidine alkaloids of *Senecio longilobus* Benth

Bad Bug Book

Foodborne Pathogenic Microorganisms and Natural Toxins

Venomous Fish

1. Introduction

Some fish produce venom in specialized spines or other structures that can cause adverse health effects in humans, from mild to lethal, if the venom is delivered through puncture wounds. However, little information is available on the potential human health consequences of consuming these fish venoms. The potential for venom contamination of fish meat during harvesting or cleaning has not been adequately investigated for any venomous fish, nor has it been established under what time, temperature, and/or pH conditions fish venoms are inactivated during cooking.

While the vast majority of commercially and recreationally harvested fish species are not venomous, these unknowns in a few species represent potential food-safety issues. For example, lionfish (*Pterois volitans*), a known venomous species from the Pacific Ocean, recently has become invasive and over-abundant along the U.S. south Atlantic coast and in the waters surrounding several Caribbean island countries, presenting new opportunities for human consumption. Currently FDA has no specific guidance for seafood processors as to the control of hazards from fish venom. As noted, the potential for harm from consuming this and any of the other known venom-producing fish species has not been adequately investigated.

2. Venomous Species

Venom-containing spines have been documented in species from primitive cartilaginous fish, such as stingrays, to

For Consumers: Lionfish in the News

Lionfish have sharp spines on their fins that can cause injury to humans and release venom (poison) if a person picks up or steps on one of these fish. The venom mainly causes pain, but, in rare cases, also can cause other complications, such as low blood pressure and temporary paralysis. Lionfish are native to the Pacific and recently have been introduced into Atlantic and Caribbean waters, where they are spreading quickly. These fish have been in the news because their numbers are rapidly growing along the southeastern U.S. coast and around some Caribbean islands; in other words, they have become "invasive." Although widespread fishing of these fish could help reduce their numbers, so that they don't crowd out other kinds of ocean life, not enough is known about whether eating their meat can cause harm. To date, no illnesses from eating lionfish have been reported, but this might not mean that there have been no illnesses. (People often don't report illnesses of many kinds to their doctors.) Scientists need to do research before it will be known if eating lionfish can cause harm. For example, it's not known if lionfish venom can get into the flesh of the fish while they're caught or cleaned, and whether it can cause illness or an allergic reaction when the fish are eaten. It's also not known if cooking or freezing fully inactivates the venom (makes it harmless).

Another issue is that lionfish are at the top of the food chain in tropical waters; in other words, they eat fish and other creatures that have eaten others, that have eaten others, and so on. In areas where other poisons called ciguatoxins are common in ocean creatures, the ciguatoxins can build up in lionfish that eat those creatures. To the FDA's knowledge, no cases of human poisoning from ciguatoxin have been linked to eating lionfish. Lionfish are not in the FDA's guidance about seafood safety, at this time, but the FDA is gathering more data about the safety of eating them, including whether ciguatoxin build-up in lionfish can, if eaten, harm people.

more advanced, bony fish such as scorpionfish, stonefish, weeverfish, blennies, and, as noted, lionfish. Venom injections from certain stonefish species (*Synanceja horrida, S. trachynis,* and *S. verrucosa*) are the most notorious among venomous fish, and have been responsible for numerous deaths from incidents in coastal Indo-Pacific waters.

Several venomous fish species are commercially and recreationally harvested for human consumption, including stingrays, marine catfish, and scorpionfish. In addition, many venomous fish species are commonly sold in the home aquarium trade, and numerous stings have been documented from the handling of these fish.

Venomous fish are found in diverse habitats, from freshwater streams to coral reefs to the open ocean. The greatest variety is found in the waters surrounding Indo-Pacific island countries, eastern and southern Africa, Australia, Polynesia, the Philippines, Indonesia, and southern Japan. Most venomous fish inhabit shallow, inshore waters among coral reefs and rocks. They generally swim slowly and are non-migratory, and tend either to be brightly colored or to blend in with their environments. Stonefish, as their name suggests, are well camouflaged in their native habitat, and most lethal envenomations have occurred through accidental contact (i.e., being stepped on) by recreational divers and fishermen.

Several venomous fish species are top predators in tropical coral-reef food chains and, therefore, have the potential to accumulate ciguatoxins in their flesh and internal organs in ciguatera-endemic areas and cause poisoning. *Ciguatoxins cannot be removed during processing or deactivated through cooking*. The FDA has issued guidance, in the Fish and Fisheries Products Hazards and Controls Guidance, on avoiding seafood species known to cause ciguatera from endemic regions. For more information on ciguatoxins, see the chapter on Ciguatera Fish Poisoning in this publication and the natural toxins chapter in the FFPHG. No venomous species are currently listed as hazardous to consumers from ciguatera in the FFPHG; however, additional species are included as new data are gathered.

3. Fish Venom

Fish venoms are complex mixtures of proteins and enzymes, each with its own biological activity, most of which have yet to be isolated and characterized. Studies have shown that many fish venoms are chemically and pharmacologically similar.

Fish venoms are known to have cardiovascular, neuromuscular, inflammatory, and cytolytic properties. No fish venom mixtures have been fully characterized, and only a few components (e.g. stonustoxin, a lethal compound from the stonefish *Synanceja horrida*, which causes severe hypotension) have been purified and studied in detail. Although fish venoms are believed to be unstable and heat labile, no thorough studies have been performed on the potency of venom components after fish harvest or death.

4. Venom Apparatus in Fish

Fish venom is produced in specialized glands associated with distinct venom-delivery structures. Most of these structures are spines located on the dorsal (back), pectoral, pelvic, anal, caudal (tail) or opercular (cheek) surfaces. The venom-producing glands are usually located in a groove on the surface or at the base of the spine. The size and complexity of this glandular tissue varies by species. Unlike other venomous creatures, such as spiders, wasps, and snakes, in which venom can be actively injected through a bite or sting, fish venom is delivered involuntarily

when a spine pierces the tissue of the victim, leading to rupture of the spine's sheath, and venom passes into the puncture wound.

5. Symptoms

No information is available on the occurrence or potential health consequences of consuming fish venom. Around the world, numerous cases of fish stings have been reported from both commercial and recreational fisherman attempting to harvest venomous fish species. In terms of envenomation by puncture, the severity of symptoms depends on the fish species, amount of venom delivered, and age and health status of the victim. The most common symptom associated with envenomation by puncture is acute, localized pain disproportionate to the size or severity of the wound. This symptom reaches its greatest intensity within 60 to 90 minutes and, if untreated, can last 8 to 12 hours.

In addition to the localized symptoms and complications associated with the puncture wound itself, systemic symptoms occur in a limited number of victims. They include dizziness, nausea or vomiting, difficulty breathing, chest pain, abdominal pain, hypotension, and generalized weakness. Stonefish envenomations appear to be the most potent and may result in death from hypotension, arrhythmia, and/or pulmonary edema.

A secondary consequence of handling fish with venomous spines is bacterial infection of the wound, particularly from species with barbed spines (e.g. catfish, stingrays) that can break off and become embedded in the victim. Medical attention should be sought in cases in which the spines cannot be removed or systemic symptoms persist.

6. Treatment

As this book concerns foodborne illnesses, treatment for the puncture wounds themselves will not be discussed in detail. The most common and effective treatment for acute pain from fish envenomation is immersion of the affected area in hot (45°C, not boiling) water for as long as is tolerable by the patient. Tetanus or antibiotic treatment may be administered by a health professional, if secondary infection of the wound is suspected. For severe cases of stonefish envenomation, commercial antivenom is available. In laboratory studies, this product has been shown to be effective in reducing the potency of several scorpionfish venoms, including those from the devil stinger (*Inimicus japonicus*), lionfish (*Pterois volitans*, *P. lunulata*, and *P. antennata*), and zebra turkeyfish (*Dendrochirus zebra*).

7. Resources

CDC/MMWR (venom AND fish): CDC's Morbidity and Mortality Weekly Report.

NIH/PubMed (venom AND fish): Research abstracts in the National Library of Medicine's MEDLINE database.

Agricola (venom AND fish) Research abstracts in the USDA NAL database.

8. Photos of Venomous Fish

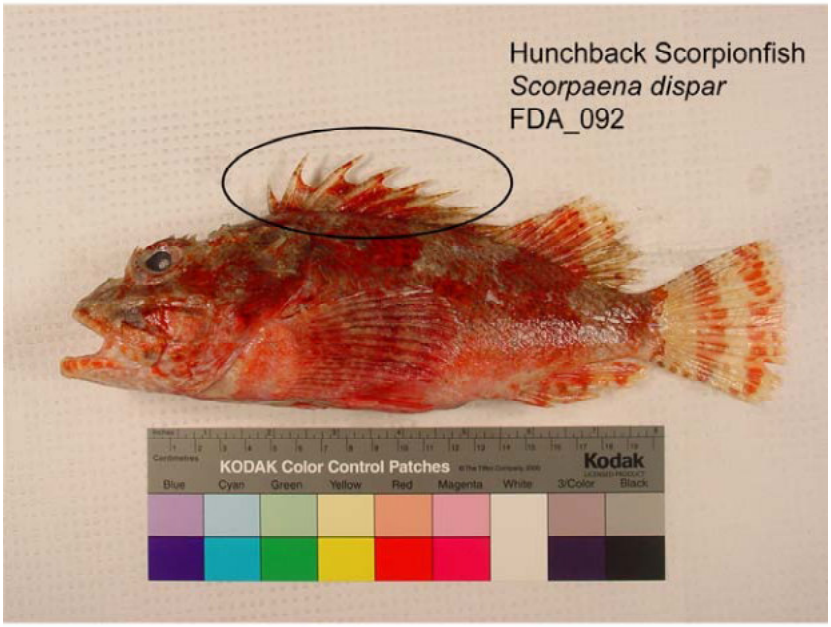

Photo by Jonathan Deeds, Ph.D., FDA

Photo by Jonathan Deeds, Ph.D., FDA

Grayanotoxins

1. Toxin

Grayanotoxin is found in the leaves, flowers, and nectar of some *Rhododendron* species and from other members of the Ericaceæ botanical family. (In the past, names used for this toxic chemical included andromedotoxin, acetylandromedol, and rhodotoxin.) It is also known to be present in honey produced from the pollen and nectar of certain plants in this family; particularly in honey associated with certain *Rhododendron* plants. The specific type of grayanotoxin compound(s) varies with the plant species. These toxic compounds are diterpenes, polyhydroxylated cyclic hydrocarbons that do not contain nitrogen. (See Section 11, below, for chemical structures Grayanotoxin GI-IV).

2. Toxic reaction / disease

The principal poisoning associated with exposure to grayanotoxin is known as "honey intoxication." It is most often associated with consumption of honey produced from the pollen and nectar of rhododendrons. Toxic concentrations of grayanotoxin cause adverse reaction(s). Other names for this toxicity are rhododendron poisoning, mad honey intoxication, and grayanotoxin poisoning.

For Consumers: A Snapshot

If bees make their honey from the pollen and nectar of flowers from some types of rhododendron, the honey may contain grayanotoxin, a substance poisonous to humans. Other plants from the same family that may contain it, in the Eastern part of the U.S., include mountain laurel and sheep laurel. Sickness that results from eating honey that contains grayanotoxin is sometimes called "mad honey" poisoning. It has occurred in the past in the U.S., but now appears to be very rare here. In other countries, however, some honeys imported from Turkey have recently caused mad honey sickness. The signs and symptoms (described below) start soon after the honey is eaten, within minutes to a couple of hours, and are gone within a day or so, except in the more severe cases. Honey that contains grayanotoxin may be brown and bitter and may cause a burning feeling in the throat.

Honey produced by large businesses in the U.S. often consists of huge amounts pooled together from a variety of sources, so any toxin that might be present would be diluted to tiny amounts not likely to be harmful. If you have any concerns about the honey you're buying from a local bee-keeper, ask questions, to see if the person knows about the toxin and about what kinds of flowers live in the area where his or her bees collect pollen.

Nausea and vomiting are common symptoms of grayanotoxin poisoning. A rarer symptom is burning, tingling, and numbness around the mouth. The toxin affects nerve cells, including not only the nerves that affect the brain, but also those that affect the heart and other muscles. For this reason, grayanotoxin poisoning causes not only problems like dizziness, weakness, confusion, vision disturbances, and heavy sweating and saliva flow, but also irregular or very slow heartbeat, low blood pressure, and fainting. These poisonings are rarely fatal. Even in cases of severe poisoning, medical treatments can counteract the toxic effect; for example, they can help keep the blood pressure and heart rate from becoming dangerously low.

- **Mortality:** This type of food poisoning is rarely fatal, even in severe cases, if appropriate medical treatment is administered in a timely manner.

- **Toxic dose:** The lowest dose is reported to be between 5 g and 30 g, but the amounts vary and have ranged as high as about 300 g. However, it should be kept in mind that vomiting is a very common symptom of exposure to grayanotoxin and may alter the actual dose and the amount of toxin absorbed. The occurrence or severity of honey poisoning has not been related to the amount of honey ingested, in studies that attempted to directly evaluate the relationship (Yilmaz *et al*., 2006; Gunduz *et al*., 2006), although some references have suggested that this may be the case. The concentration of grayanotoxin in the honey ingested probably is a significant factor. It is thought to vary greatly across honey product, but rarely is measured.

- **Onset:** Symptoms of poisoning occur after a few minutes to 2 or more hours. It has been suggested that the latent period for symptom onset is dose-dependent (e.g., Gunduz *et al.*, 2006), but no association between amount of honey eaten and symptom onset was seen in a study that directly examined the relationship (Yilmaz *et al*., 2006).

- **Illness / treatment:** Grayanotoxins are neurotoxins and cardiotoxins. In mild cases, recovery generally occurs within about 2 to 8 hours, and intervention may not be required. In cases in which severe adverse reactions are seen, low blood pressure usually responds to administration of fluids and correction of bradycardia; therapy with vasopressors may be required. Sinus bradycardia and conduction defects usually respond to atropine therapy. Recovery in these intoxication cases usually occurs within 24 hours. However, some severely poisoned people require care and monitoring in (coronary) intensive-care units for several days prior to recovery. In at least a few instances, use of a temporary pacemaker has been required. Under the circumstances described, the outcome of mad honey intoxication is rarely fatal.

- **Symptoms:** The adverse reaction induced by grayanotoxins includes nausea and vomiting; dizziness; weakness; mental confusion or impaired consciousness; excessive perspiration and/or salivation, cloudy or blurred vision; chest pain or compression; paresthesias in the extremities or perioral area shortly after the toxic honey is ingested. Cardiovascular effects may include fainting, low blood pressure or shock, bradyarrhythmia (slow, irregular heartbeat), sinus bradycardia (regular heart rhythm, but with rate slower than 60 beats per minute), and abnormalities in the heart's pacemaker / conduction pathways (e.g., nodal rhythm, second degree or complete atrioventricular block). Another cardiac complication reported was an occurrence of acute myocardial infarction (with normal coronary arteries) due to coronary hypoperfusion.

- **Duration:** Generally within about 24 hours, especially when treatment is promptly administered in more serious cases. Because grayanotoxins are metabolized and excreted rapidly, patients typically feel better and experience an alleviation of grayanotoxin-induced symptoms along with a return to normal cardiac function, as seen in measures such as heart and blood pressure, within a relatively brief duration. In mild poisonings, the duration of adverse effects are typically a few hours; in severe cases, the duration of the effects can be 1 to 5 days.

- **Route of entry:** Oral.

- **Pathway / mechanism:** The responses of skeletal and heart muscle, peripheral nerves, and the central nervous system are related to effects of grayanotoxin on the cell membrane. The grayanotoxins bind to voltage-gated sodium channels in cell membranes, causing the channels to open at lower-than-normal membrane potentials and to remain open more than usual. The resulting increase in sodium influx and sustained depolarization cause hyperexcitability of the cell. Entry of calcium into the cells also may be facilitated during this time.

3. Frequency

Occurrence of honey intoxication has been sporadic. The toxic reaction has occurred more often in certain geographical locations, with the Black Sea area of Turkey being the predominant one. It may be more likely in springtime, because honey produced during this season tends to have a higher concentration of grayanotoxin than does honey from other seasons. In addition, honey obtained from farmers who may have only a few hives is associated with an increased risk of a honey intoxication reaction. In contrast, the pooling of massive quantities of honey during commercial processing generally serves to dilute the amount of any toxic substance. So-called "mad honey" may be distinguished by its brown color, linden-flower smell and bitter taste, along with the sharp, burning sensation it may cause in the throat.

4. Sources

Grayanotoxin poisoning most commonly results from ingestion of grayanotoxin-contaminated honey, although it may result from ingestion of components of the plants in the Ericaceæ family or their use as a tea. Not all rhododendrons produce grayanotoxins. The species that has been associated with honey poisoning since 401 BC is the *Rhododendron ponticum* and *luteum*. It grows extensively on the mountains of the eastern Black Sea area of Turkey. A number of toxic species are native to the United States. Of particular importance are the western azalea (*Rhododendron occidentale*), found from Oregon to southern California; the California rosebay (*Rhododendron macrophyllum*), found from British Columbia to central California; and *Rhododendron albiflorum*, found from British Columbia to Oregon and in Colorado. In the eastern half of the U.S., grayanotoxin-contaminated honey may be derived from other members of the botanical family Ericaceæ. This includes the mountain laurel (*Kalmia latifolia*) and sheep laurel (*Kalmia angustifolia*), which probably are the other most important sources of the toxin.

5. Diagnosis

Diagnosis is by the evaluation of characteristic signs and symptoms of grayanotoxin intoxication, along with the assessment of recent consumption behavior and choices of the patient. No blood or urine tests are readily available.

6. Target populations

Although human grayanotoxin poisoning from honey is rare, all people are believed to be susceptible, and cases may occur anywhere that honey is consumed. Added vulnerability or altered outcome are a possibility among people with pre-existing cardiovascular disease or blood-pressure issues. Grayanotoxin poisonings in Germany, Austria, and Korea have been attributed to honey from Turkey. Consumption of "mad honey" as an alternative medicinal or "natural" therapy for an illness or to improve health, or as a folk cure, has been noted in the literature. Grayanotoxin poisonings also are common in livestock, particularly in sheep and goats fed with the young leaves or flowers of certain rhododendron species.

7. Food Analysis

The grayanotoxins can be isolated from the suspect commodity by typical extraction procedures for naturally occurring terpenes. The toxins can be identified by thin-layer chromatography (Scott, *et al*., 1971; Froberg *et al*., 2007).

8. Examples of cases

See Resources section, below.

9. Resources

- CDC/MMWR: Grayanotoxin – Provides a list of Morbidity and Mortality Weekly Reports, from the Centers for Disease Control and Prevention (CDC), relating to this toxin. At the time of this writing, a search of the term "grayanotoxin" resulted in no current reports of grayanotoxin poisoning in CDC's MMWR. However, if such reports should emerge, they would appear at the link above, which readers may check periodically.

- TOXNET – Toxicology Data Network, from the National Library of Medicine.

- NIH/PubMed: Grayanotoxin Provides a list of research abstracts contained in the National Library of Medicine's MEDLINE database.

- Agricola: Grayanotoxin – Provides a list of research abstracts contained in the National Agricultural Library database.

- Loci index for genome *Rhododendron* spp. (Available from the GenBank Taxonomy database).

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Additional educational and background resources

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11. Molecular Structural Data:

There are four principle toxic isomers of grayanotoxin, designated as I, II, III, and IV, in plants from the Ericaceæ botanical family.

Grayanotoxins GI-IV

Phytohaemagglutinin (kidney bean lectin)

For Consumers: A Snapshot

Beans are a great deal, nutrition-wise and cost-wise – but be sure to cook your kidney beans well. If you eat them raw or under-cooked, they can cause you to have extreme nausea, severe vomiting, and diarrhea. They contain a protein that's found naturally in many plants (and animals, including humans), where it performs important functions. But when it reaches high levels in some plants, particularly kidney beans, the protein can act as a toxin. Cooking the beans properly destroys the toxin. *Don't* use slow cookers (the kinds of pots that you plug in and that cook food at low temperatures for several hours) to cook these beans or dishes that contain them. Slow cookers don't get hot enough to destroy the toxin in kidney beans. Studies done by British scientists suggest that beans should be soaked in water for at least 5 hours, the water poured away, and the beans boiled in fresh water for at least 30 minutes.

1. Protein / Toxin

Lectins are widely occurring, sugar-binding proteins that perform a variety of biological functions in plants and animals, including humans, but some of them may become toxic at high levels. Besides inducing mitosis, lectins are known for their ability to agglutinate many mammalian red blood cell types, alter cell-membrane transport systems, alter cell permeability to proteins, and generally interfere with cellular metabolism.

Among the lectins known to have toxic effects is phytohaemagglutinin, which occurs at relatively high levels in the seeds of legumes (e.g., beans). The role of this compound in defense against plant pests and pathogens has been established.

This hemagglutinin also is used in research; for example, to trigger DNA and RNA synthesis in T lymphocytes, *in vitro*. PHAs are used to test competence of cell-mediated immunity; for example, in patients with chronic viral infections.

2. Disease

Red kidney bean (*Phaseolus vulgaris*) poisoning and kinkoti bean poisoning are examples of names for the illness caused by phytohaemagglutinin.

- **Mortality:** not reported.
- **Toxic dose:** As few as four or five raw beans can trigger symptoms.
- **Onset:** Usually begins with extreme nausea and vomiting within 1 to 3 hours of ingestion of the product, with diarrhea developing later within that timeframe.
- **Illness / complications:** Upper and lower gastrointestinal illness. Vomiting may become severe.
- **Symptoms:** In addition to vomiting and diarrhea, abdominal pain has been reported by some people.

- **Duration:** Recovery usually is rapid, within 3 to 4 hours after onset of symptoms, and spontaneous, although some cases have required hospitalization.

- **Route of entry:** Oral (consumption of uncooked or undercooked kidney beans).

- **Pathway:** The mechanism and pathway of toxicity is not known, but oral ingestion of lectins is known to reduce intestinal absorption and cause weight loss, growth retardation, and diarrhea in several animal species.

3. Frequency

This syndrome has occurred in the United Kingdom with some regularity. Seven outbreaks occurred in the U.K. between 1976 and 1979. Two more incidents were reported by the Public Health Laboratory Services (PHLS), of Colindale, U.K., in the summer of 1988. Reports of this syndrome in the United States are anecdotal and have not been formally published.

4. Sources

Phytohaemagglutinin, the presumed toxic agent, is found in many species of beans, but is in highest concentration in red kidney beans (*Phaseolus vulgaris*). The unit of toxin measure is the hemagglutinating unit (hau). Raw kidney beans contain from 20,000 to 70,000 hau, while fully cooked beans contain from 200 to 400 hau. White kidney beans, another variety of *Phaseolus vulgaris*, contain about one-third the amount of toxin as the red variety; broad beans (*Vicia faba*) contain 5% to 10% the amount that red kidney beans contain.

The syndrome usually is caused by ingestion of raw, soaked kidney beans, either alone or in salads or casseroles. Several outbreaks have been associated with beans cooked in slow cookers (i.e., countertop appliances that cook foods at low temperatures for several hours) or in casseroles that had not reached an internal temperature high enough to destroy the glycoprotein lectin.

PHA is destroyed by adequate cooking. Some variation in toxin stability has been found at different temperatures. However, Bender and Readi found that boiling the beans for 10 minutes (100°C) completely destroyed the toxin. Consumers should boil the beans for at least 30 minutes to ensure that the product reaches sufficient temperature, for a sufficient amount of time, to completely destroy the toxin. Slow cookers should not be used to cook these beans or dishes that contain them. Studies of casseroles cooked in slow cookers revealed that the food often reached internal temperatures of only 75°C or less, which is inadequate for destruction of the toxin.

5. Diagnosis

Diagnosis is made on the basis of symptoms, food history, and exclusion of other rapid-onset food-poisoning agents (e.g., *Bacillus cereus*, *Staphylococcus aureus*, arsenic, mercury, lead, and cyanide).

6. Target Populations

All people, regardless of age or gender, appear to be equally susceptible; the severity is related to the dose ingested. In the seven outbreaks mentioned below, the attack rate was 100%.

7. Food Analysis

The difficulty in food analysis is that this syndrome is not well known in the medical community. Other possible causes, such as *Bacillus cereus*, staphylococcal food poisoning, and chemical toxicity, must first be eliminated. If beans were a component of the suspect meal, analysis is quite simple, based on hemagglutination of red blood cells (hau).

8. Examples of Outbreaks

- Article: Food Poisoning from Raw Red Kidney Beans (Noah, Bender, *et al.* 1980)
- Article: Red Kidney Bean Poisoning in the UK: An Analysis of 50 Suspected Incidents Between 1976 and 1989. (Rodhouse, Haugh, *et al*., 1990)
- Agricola: Phytohaemagglutinin – Provides a list of research abstracts contained in the National Agricultural Library database.
- CDC Morbidity and Mortality Weekly Reports.

9. Resources

- Loci index for genome *Phaseolus vulgaris*
- GenBank Taxonomy database

10. Molecular Structural Data

Phytohaemagglutinin Structural Information Database and Image

Appendices

Appendix 1. Infective Dose Information

Most chapters include a statement on the infective dose necessary to cause disease. These numbers should be viewed with caution for any of the following reasons:

- Often they were extrapolated from epidemiologic outbreak investigations which, at best, give a very rough estimate of infectious dose.
- They were obtained by human feeding studies on healthy, young adult volunteers who may be less susceptible to infection than are young children, older adults, or immunocompromised people.
- They may represent a higher estimate of the actual infective dose.

There are many variables that impact how many cells of a pathogen are needed to cause illness. While the infective dose numbers provided in the BBB chapters represent the best current thinking, results of future research may alter the knowledge base. Variables that can impact an infective dose include the following:

Variables of the Parasite or Microorganism

- Variability of gene expression of multiple pathogenic mechanism(s)
- Potential for damage or stress of the microorganism
- Interaction of organism with food menstruum and environment
- pH susceptibility of organism
- Immunologic "uniqueness" of the organism
- Interactions with other organisms

Variables of the Host

- Age
- General health
- Pregnancy
- Medications – OTC or prescription
- Metabolic disorders
- Alcoholism, cirrhosis, hemochromatosis
- Malignancy treatment
- Amount of food consumed (number of cells consumed)
- Gastric acidity variation: antacids, natural variation, achlorhydria
- Genetic disturbances
- Nutritional status
- Immune competence
- Surgical history
- Occupation

Appendix 2. From the CDC: Summaries of selected estimates

The Centers for Disease Control and Prevention estimate that, each year, roughly 1 of 6 Americans (48 million people) get sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases. The 2011 estimates provide the most accurate picture yet of which foodborne bacteria, viruses, microbes ("pathogens") are causing the most illnesses in the United States, and include the number of foodborne illnesses without a known cause.* The estimates show that there is still much work to be done—specifically in focusing efforts on the top known pathogens and identifying the causes of foodborne illness and death without a known cause.

CDC has estimates for two major groups of foodborne illnesses:

Known foodborne pathogens – 31 pathogens known to cause foodborne illness. Many of these pathogens are tracked by public health systems that track diseases and outbreaks.

***Unspecified agents** – Agents with insufficient data to estimate agent-specific burden; known agents not yet identified as causing foodborne illness; microbes, chemicals, or other substances known to be in food whose ability to cause illness is unproven; and agents not yet identified. Because you can't "track" what isn't yet identified, estimates for this group of agents started with the health effects or symptoms that they are most likely to cause—acute gastroenteritis.

To estimate the total number of foodborne illnesses, CDC estimated the number of illnesses caused by both known and unspecified agents and estimated the number of hospitalizations and deaths they caused. Table 1 provides the estimates due to known pathogens, unspecified agents, and the total burden. Table 2 provides estimates of the top five pathogens that cause domestically acquired foodborne illness in the U.S.

Table 1. Estimated annual number of domestically acquired foodborne illnesses, hospitalizations, and deaths due to 31 pathogens and unspecified agents transmitted through food, United States

Foodborne agents	Estimated annual number of illnesses (90% credible interval)	%	Estimated annual number of hospitalizations (90% credible interval)	%	Estimated annual number of deaths (90% credible interval)	%
31 known pathogens	9.4 million (6.6–12.7 million)	20	55,961 (39,534–75,741)	44	1,351 (712–2,268)	44
Unspecified agents	38.4 million (19.8–61.2 million)	80	71,878 (9,924–157,340)	56	1,686 (369–3,338)	56
Total	47.8 million (28.7–71.1 million)	100	127,839 (62,529–215,562)	100	3,037 (1,492–4,983)	100

Table 2. Top five pathogens causing domestically acquired foodborne illnesses, United States

Pathogen	**Estimated annual number of illnesses**	**90% Credible Interval**	**%**
Norovirus	5,461,731	3,227,078–8,309,480	58
Salmonella, nontyphoidal	1,027,561	644,786–1,679,667	11
Clostridium perfringens	965,958	192,316–2,483,309	10
Campylobacter spp.	845,024	337,031–1,611,083	9
Staphylococcus aureus	241,148	72,341–529,417	3
	Subtotal		91

Source: www.cdc.gov/foodborneburden

Appendix 3. Factors that Affect Microbial Growth in Food

- Bacteriological Analytical Manual

Food is a chemically complex matrix. Predicting whether, or how fast, microorganisms will grow in a food is difficult. Most foods contain sufficient nutrients to support microbial growth. Several factors encourage, prevent, or limit growth of microorganisms in foods; the most important are a_w, pH, and temperature. These factors can be divided into two broad categories: intrinsic and extrinsic factors. Intrinsic factors are inherent to food, such as a_w and pH. Extrinsic factors are external conditions under which food is stored that affect microbial growth in foods, such as temperature and relative humidity.

a_w: (Water Activity or Water Availability). Water molecules are loosely oriented in pure liquid water and can easily rearrange. When other substances (solutes) are added to water, water molecules orient themselves on the surface of the solute, and the properties of the solution change dramatically. The microbial cell must compete with solute molecules for free water molecules. Except for *Staphylococcus aureus*, bacteria are rather poor competitors, whereas molds are excellent competitors.

The a_w varies very little with temperature over the range of temperatures that support microbial growth. A solution of pure water has an a_w of 1.00. The addition of solute decreases the a_w to less than 1.00.

Water Activity of Various NaCl Solutions		
Percent NaCl (w/v)	**Molal**	**Water Activity (a_w)**
0.9	0.15	0.995
1.7	0.30	0.99
3.5	0.61	0.98
7.0	1.20	0.96
10.0	1.77	0.94
13.0	2.31	0.92
16.0	2.83	0.90
22.0	3.81	0.86

The **a_w** of a solution may dramatically affect the ability of heat to kill a bacterium at a given temperature. For example, a population of *Salmonella* Typhimurium is reduced 10-fold in 0.18 minutes at 60°C, if the a_w of the suspending medium is 0.995. If the a_w is lowered to 0.94, the same 10-fold reduction requires 4.3 min at 60°C.

An **a_w** value stated for a bacterium is generally the minimum a_w that supports growth. At the minimum a_w, growth is usually minimal, increasing as the a_w increases. At a_w values below the minimum for growth, bacteria do not necessarily die, although some proportion of the population does die. The bacteria may remain dormant, but infectious. Most importantly, a_w is only one factor, and the other factors (e.g., pH, temperature) of the food must be considered. It is the interplay between factors that ultimately determines if a bacterium will grow or not. The a_w of a

food may not be a fixed value; it may change over time, or may vary considerably between similar foods from different sources.

pH: (hydrogen ion concentration, relative acidity or alkalinity). The pH range of a microorganism is defined by a minimum value (at the acidic end of the scale) and a maximum value (at the basic end of the scale). There is a pH optimum for each microorganism at which growth is maximal. Moving away from the pH optimum in either direction slows microbial growth.

A range of pH values is presented here, as the pH of foods, even those of similar types, varies considerably. Shifts in pH of a food with time may reflect microbial activity, and foods that are poorly buffered (i.e., do not resist changes in pH), such as vegetables, may shift pH values considerably. For meats, the pH of muscle from a rested animal may differ from that of a fatigued animal.

A food may start with a pH that precludes bacterial growth, but as a result of the metabolism of other microbes (yeasts or molds), pH shifts may occur and permit bacterial growth.

pH Values of Various Foods

BAKERY PRODUCTS	pH
Bread	5.3 - 5.8
Éclairs	4.4 - 4.5
Napoleons	4.4 - 4.5
Biscuits	7.1 - 7.3
Crackers	7.0 - 8.5
Cakes, Angel food	5.2 - 5.6
Cakes, Chocolate	7.2 - 7.6
Cakes, Devil's food	7.5 - 8.0
Cakes, Pound	6.6 - 7.1
Cakes, Sponge	7.3 - 7.6
Cakes, White layer	7.1 - 7.4
Cakes, Yellow layer	6.7 - 7.1
Flour	6.0 - 6.3
BERRIES	**pH**
Blackberries	3.2 - 4.5
Blueberries	3.7
Blueberries, Frozen	3.1 - 3.35
Cherries	3.2 - 4.1
Cranberries, Sauce	2.4
Cranberries, Juice	2.3 - 2.5
Currants (red)	2.9
Gooseberries	2.8 - 3.1
Grapes	3.4 - 4.5
Raspberries	3.2 - 3.7
Strawberries	3.0 - 3.5
Strawberries, Frozen	2.3 - 3.0
DAIRY PRODUCTS/ EGGS	**pH**
Butter	6.1 - 6.4
Buttermilk	4.5
Milk	6.3 - 8.5
Acidophilus	4.0

Cream	6.5
Cheese, Camembert	7.4
Cheese, Cheddar	5.9
Cheese, Cottage	5.0
Cheese, Cream cheese	4.88
Cheese, Edam	5.4
Cheese, Roquefort	5.5 - 5.9
Cheese, Swiss Gruyere	5.1 - 6.6
Eggs, White	7.0 - 9.0
Eggs, Yolk	6.4
Egg solids, whites	6.5 - 7.5
Eggs, Whole	7.1 - 7.9
Eggs, Frozen	8.5 - 9.5
FISH	**pH**
Fish (most fresh)	6.6 - 6.8
Clams	6.5
Crabs	7.0
Oysters	4.8 - 6.3
Tuna fish	5.2 - 6.1
Shrimp	6.8 - 7.0
Salmon	6.1 - 6.3
Whitefish	5.5
Freshwater (most)	6.9 - 7.3
Sturgeon	5.5 - 6.0
Herring	6.1 - 6.4
Fruits	**pH**
Apples, Delicious	3.9
Apples, Golden Delicious	3.6
Apples, Jonathan	3.33
Apple, McIntosh	3.34
Apple, Winesap	3.47
Apple, Juice	3.4 - 4.0

Apple, Sauce	3.3 - 3.6
Apricots	3.3 – 4.0
Apricots, Dried	3.6 - 4.0
Apricots, Canned	3.74
Bananas	4.5 - 5.2
Cantaloupe	6.17-7.13
Dates	6.3 - 6.6
Figs	4.6
Grapefruit	3.0 - 3.3
Grapefruit, Canned	3.1 - 3.3
Grapefruit, Juice	3.0
Lemons	2.2 - 2.4
Lemons, Canned juice	2.3
Limes	1.8 - 2.0
Mangos	3.9 - 4.6
Melons, Casaba	5.5 - 6.0
Melons, Honeydew	6.3 - 6.7
Melons, Persian	6.0 - 6.3
Nectarines	3.9
Oranges	3.1 - 4.1
Oranges, Juice	3.6 - 4.3
Oranges, Marmalade	3.0
Papaya	5.2 - 5.7
Peaches	3.4 - 3.6
Peaches, In jars	4.2
Peaches, In cans	4.9
Persimmons	5.4 - 5.8
Pineapple	3.3 - 5.2
Pineapple, Canned	3.5
Pineapple, Juice	3.5
Plums	2.8 - 4.6
Pomegranates	3.0
Prunes	3.1 - 5.4
Prunes, Juice	3.7
Quince (stewed)	3.1 - 3.3
Tangerines	4.0
Watermelon	5.2 - 5.8
Meat, Poultry	**pH**
Beef, Ground	5.1 – 6.2
Beef, Ripened	5.8
Beef, Unripened	7.0
Beef, Canned	6.6
Beef, Tongue	5.9
Ham	5.9 - 6.1
Lamb	5.4 - 6.7
Pork	5.3 - 6.9
Veal	6.0
Chicken	6.5 – 6.7
Turkey (roasted)	5.7 – 6.8
VEGETABLES	**pH**
Artichokes	5.6
Artichokes, Canned	5.7 – 6.0
Asparagus	4.0 – 6.0
Asparagus, Canned	5.2 - 5.3

Asparagus, Buds	6.7
Asparagus, Stalks	6.1
Beans	5.7 - 6.2
Beans, String	4.6
Beans, Lima	6.5
Beans, Kidney	5.4 – 6.0
Beets	4.9 - 5.6
Beets, Canned	4.9
Brussel sprouts	6.0 - 6.3
Cabbage	5.2 - 6.0
Cabbage, Green	5.4 - 6.9
Cabbage, White	6.2
Cabbage, Red	5.4 - 6.0
Cabbage, Savoy	6.3
Carrots	4.9 - 5.2
Carrots, Canned	5.18-5.22
Carrots, Juice	6.4
Cauliflower	5.6
Celery	5.7 - 6.0
Chives	5.2 - 6.1
Corn	6.0 - 7.5
Corn, Canned	6.0
Corn, Sweet	7.3
Cucumbers	5.1 - 5.7
Dill pickles	3.2 - 3.5
Eggplant	4.5 - 5.3
Hominy (cooked)	6.0
Horseradish	5.35
Kale (cooked)	6.4 - 6.8
Kohlrabi (cooked)	5.7 - 5.8
Leeks	5.5 - 6.0
Lettuce	5.8 - 6.0
Lentils (cooked)	6.3 - 6.8
Mushrooms (cooked)	6.2
Okra (cooked)	5.5 - 6.4
Olives, Green	3.6 - 3.8
Olives, Ripe	6.0 - 6.5
Onions, Red	5.3 - 5.8
Onions, White	5.4 - 5.8
Onions, Yellow	5.4 - 5.6
Parsley	5.7 - 6.0
Parsnip	5.3
Peas	5.8 - 7.0
Peas, Frozen	6.4 - 6.7
Peas, Canned	5.7 - 6.0
Peas, Dried	6.5 - 6.8
Pepper	5.15
Pimiento	4.6 - 4.9
Potatoes	6.1
Potatoes, Tubers	5.7
Potatoes, Sweet	5.3 - 5.6
Pumpkin	4.8 - 5.2
Radishes, Red	5.8 - 6.5
Radishes, White	5.5 - 5.7

Rhubarb	3.1 - 3.4
Rhubarb, Canned	3.4
Rice, Brown (cooked)	6.2 - 6.7
Rice, White (cooked)	6.0 - 6.7
Rice, Wild (cooked)	6.0 - 6.4
Sauerkraut	3.4 - 3.6
Sorrel	3.7
Spinach	5.5 - 6.8
Spinach, Cooked	6.6 - 7.2
Spinach, Frozen	6.3 - 6.5
Squash, Yellow (cooked)	5.8 - 6.0
Squash, White (cooked)	5.5 - 5.7
Squash, Hubbard (cooked)	6.0 - 6.2
Tomatoes (whole)	4.2 - 4.9
Tomato, Paste	3.5 - 4.7
Tomatoes, Canned	3.5 - 4.7

Tomato, Juice	4.1 - 4.2
Turnips	5.2 - 5.5
Zucchini (cooked)	5.8 - 6.1
MISCELLANEOUS	**pH**
Caviar (domestic)	5.4
Cider	2.9 - 3.3
Cocoa	6.3
Corn syrup	5.0
Corn starch	4.0 - 7.0
Ginger ale	2.0 - 4.0
Honey	3.9
Jams/Jellies	3.1 - 3.5
Mayonnaise	4.2 - 4.5
Molasses	5.0 - 5.5
Raisins	3.8 - 4.0
Sugar	5.0 - 6.0
Vinegar	2.0 - 3.4
Yeast	3.0 - 3.5

Temperature: Temperature values for microbial growth, like pH values, have a minimum and maximum range with an optimum temperature for maximal growth. The rate of growth at extremes of temperature determines the classification of an organism (e.g., psychrotroph, thermotroph). The optimum growth temperature determines its classification as a thermophile, mesophile, or psychrophile.

Interplay of Factors Affecting Microbial Growth in Foods

Although each of the major factors listed above plays an important role, the interplay between the factors ultimately determines whether a microorganism will grow in a given food. Often, the results of such interplay are unpredictable, as poorly understood synergism or antagonism may occur. Advantage is taken of this interplay, with regard to preventing the outgrowth of *C. botulinum*. Food with a pH of 5.0 (within the range for *C. botulinum*) and an a_w of 0.935 (above the minimum for *C. botulinum*) may not support the growth of this bacterium. Certain processed cheese spreads take advantage of this fact and are therefore shelf-stable at room temperature, even though each individual factor would permit the outgrowth of *C. botulinum*.

Therefore, predictions about whether or not a particular microorganism will grow in a food can, in general, only be made through experimentation. Also, many microorganisms do not need to multiply in food to cause disease.

Appendix 4. Foodborne Illnesses and Outbreaks: Links to Surveillance, Epidemiologic, and Related Data and Information

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- Foodborne Diseases Active Surveillance Network (FoodNet), of the Centers for Disease Control and Prevention (CDC) Emerging Infections Program. FoodNet gathers data from more than 300 laboratories throughout the country

- CDC site for trends in foodborne illness in the U.S. from 1996-2010

- Public Health Laboratory Information System (PHLIS)

- National Electronic Norovirus Outbreak Network (CaliciNet) National Molecular Subtyping Network for Foodborne Diseases Surveillance (PulseNet) uses pulsed-field gel electrophoresis (PFGE) patterns to create a database of DNA fingerprinting of several pathogens.

- National Antimicrobial Resistance Monitoring System (NARMS) monitors antimicrobial resistance of selected human bacterial pathogens.

- Foodborne Outbreak Detection Unit National Notifiable Diseases Surveillance System (NNDSS)

- National Outbreak Reporting System (NORS). CDC collects reports of foodborne outbreaks due to enteric bacterial, viral, parasitic, and chemical agents. State, local, and territorial public health agencies report these outbreaks through the National Outbreak Reporting System (NORS).

- DPDx Laboratory Identification of Parasites of Public Health Concern assists and strengthens the laboratory diagnosis of parasitic disease.

- World Health Organization surveillance site.

Appendix 5. Onset & Predominant Symptoms Associated with Selected Foodborne Organisms and Toxins

*** Note: some of the onset times listed are meant to capture only a very general sense of the timeframe. For example, the onset time under which the diarrheic form of *B. cereus* is listed in this table is 2 to 36 hours, although the *B. cereus* chapter lists onset time for this pathogen as 6 to 15 hours. The actual onset time falls within the broader timeframe listed in the table below. This structure allows organisms and toxins with similar predominant symptoms to be further grouped, in a general way. For more precise onset times, please consult each chapter.**

* Approximate onset time to symptoms	Predominant symptoms	Associated organism or toxin
Upper gastrointestinal tract symptoms occur first or predominate (nausea, vomiting)		
Less than 1 h	Nausea, vomiting, unusual taste, burning of mouth.	Metallic salts
1-2 h	Nausea, vomiting, cyanosis, headache, dizziness, dyspnea, trembling, weakness, loss of consciousness.	Nitrites
1-7 h, mean 2-4 h	Nausea, vomiting, retching, diarrhea, abdominal pain, prostration.	*Staphylococcus aureus* and its enterotoxins
0.5 to 6 h	Vomiting or diarrhea, depending on whether diarrheic or emetic toxin present; abdominal cramps; nausea.	*Bacillus cereus* (emetic toxin)
6-24 h	Nausea, vomiting, diarrhea, thirst, dilation of pupils, collapse, coma.	Amanita species mushrooms
Lower gastrointestinal tract symptoms occur first or predominate (abdominal cramps, diarrhea)		
2-36 h, mean 6-12 h	Abdominal cramps, diarrhea, putrefactive diarrhea associated with *Clostridium perfringens*; sometimes nausea and vomiting.	*Clostridium perfringens, Bacillus cereus (diarrheic form), Streptococcus faecalis, S. faecium*

12-74 h, mean 18-36 h	Abdominal cramps, diarrhea, vomiting, fever, chills, malaise, nausea, headache, possible. Sometimes bloody or mucoid diarrhea, cutaneous lesions associated with *V. vulnificus*. *Yersinia enterocolitica* mimics flu and acute appendicitis.	*Salmonella* species (including *S. arizonae*), *Shigella*, enteropathogenic *Escherichia coli*, other *Enterobacteriaceae*, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Campylobacter jejuni*, *Vibrio cholerae* (O1 and non-O1) *V. vulnificus*, *V. fluvialis*
3-5 days	Diarrhea, fever, vomiting abdominal pain, respiratory symptoms.	Enteric viruses
1-6 weeks	Diarrhea, often exceptionally foul-smelling; fatty stools; abdominal pain; weight loss.	*Giardia lamblia*
1 to several weeks	Abdominal pain, diarrhea, constipation, headache, drowsiness, ulcers, variable; often asymptomatic.	*Entamoeba histolytica*
3-6 months	Nervousness, insomnia, hunger pangs, anorexia, weight loss, abdominal pain, sometimes gastroenteritis.	*Taenia saginata, T. solium*
Neurological symptoms occur (visual disturbances, vertigo, tingling, paralysis)		
Less than 1 h	See Gastrointestinal and/or Neurological Symptoms under Shellfish Toxins in this appendix.	Shellfish toxin
	Gastroenteritis, nervousness, blurred vision, chest pain, cyanosis, twitching, convulsions.	Organic phosphate
	Excessive salivation, perspiration, gastroenteritis, irregular pulse, pupils constricted, asthmatic breathing.	Muscaria-type mushrooms
	Tingling and numbness, dizziness, pallor, gastric hemorrhage, desquamation of skin, fixed eyes, loss of reflexes, twitching, paralysis.	Tetradon (tetrodotoxin) toxins
1-6 h	Tingling and numbness, gastroenteritis, dizziness, dry mouth, muscular aches, dilated pupils, blurred vision, paralysis.	Ciguatera toxin
	Nausea, vomiting, tingling, dizziness, weakness, anorexia, weight loss, confusion.	Chlorinated hydrocarbons
2 h to 6 days, usually 12-36 h	Vertigo, double or blurred vision, loss of reflex to light, difficulty in swallowing, speaking, and breathing, dry mouth, weakness, respiratory paralysis.	*Clostridium botulinum* and its neurotoxins

More than 72 h	Numbness, weakness of legs, spastic paralysis, impairment of vision, blindness, coma.	Organic mercury
	Gastroenteritis; leg pain; ungainly, high-stepping gait; foot, wrist drop.	Triorthocresyl phosphate
Allergic symptoms occur (facial flushing, itching)		
Less than 1 h	Headache, dizziness, nausea, vomiting, peppery taste, burning of throat, facial swelling and flushing, stomach pain, itching of skin.	Histamine (scombroid)
	Numbness around mouth, tingling sensation, flushing, dizziness, headache, nausea.	Monosodium glutamate
	Flushing, sensation of warmth, itching, abdominal pain, puffing of face and knees.	Nicotinic acid
Symptoms of generalized infection occur (fever, chills, malaise, prostration, aches, swollen lymph nodes)		
4-28 days, mean 9 days	Gastroenteritis, fever, edema about eyes, perspiration, muscular pain, chills, prostration, labored breathing.	*Trichinella spiralis*
7-28 days, mean 14 days	Malaise, headache, fever, cough, nausea, vomiting, constipation, abdominal pain, chills, rose spots, bloody stools.	*Salmonella typhi*
10-13 days	Fever, headache, myalgia, rash.	*Toxoplasma gondii*
Varying periods (depends on specific illness)	Fever, chills, head- or joint ache, prostration, malaise, swollen lymph nodes, and other specific symptoms of disease in question.	*Bacillus anthracis, Brucella melitensis, B. abortus, B. suis, Coxiella burnetii, Francisella tularensis, Listeria monocytogenes, Mycobacterium tuberculosis, Mycobacterium* species, *Pasteurella multocida, Streptobacillus moniliformis, Campylobacter jejuni, Leptospira* species.
Gastrointestinal and/or neurologic symptoms - (shellfish toxins)		
0.5 to 2 h	Tingling, burning, numbness, drowsiness, incoherent speech, respiratory paralysis	Paralytic Shellfish Poisoning (PSP) (saxitoxins)

2-5 min to 3-4 h	Reversal of hot and cold sensation, tingling; numbness of lips, tongue & throat; muscle aches, dizziness, diarrhea, vomiting	Neurotoxic Shellfish Poisoning (NSP) (brevetoxins)
30 min to 2-3 h	Nausea, vomiting, diarrhea, abdominal pain, chills, fever	Diarrheic Shellfish Poisoning (DSP) (dinophysis toxin, okadaic acid, pectenotoxin, yessotoxin)
24 h (gastrointestinal) to 48 h (neurologic)	Vomiting, diarrhea, abdominal pain, confusion, memory loss, disorientation, seizure, coma	Amnesic Shellfish Poisoning (ASP) (domoic acid)

Appendix 6. Examples of International Resources

Food-safety information from New Zealand:

- http://www.foodstandards.gov.au/scienceandeducation/publications/agentsoffoodborneill5155.cfm

From the World Health Organization:

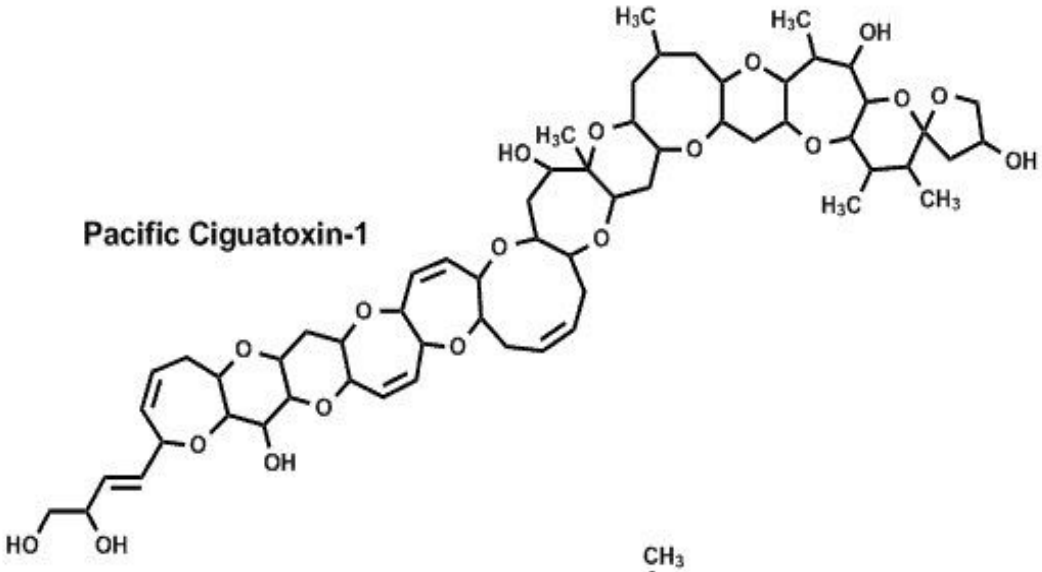
- WHO Prevention of foodborne disease: Five keys to safer food at http://www.who.int/foodsafety/consumer/5keys/en/
- WHO site for foodborne illnesses: http://www.who.int/foodsafety/foodborne_disease/en/
- WHO site for vaccine development: http://www.who.int/vaccine_research/en/
- Initiative to estimate the Global Burden of Foodborne Diseases http://www.who.int/foodsafety/foodborne_disease/ferg/en/
- WHO site for burden of foodborne disease at http://www.who.int/foodborne_disease/burden/en/

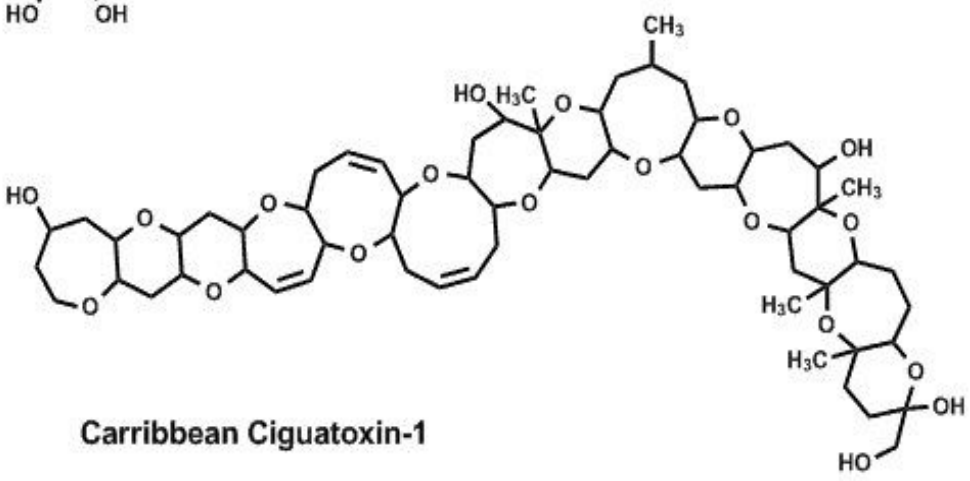
Appendix 7. Toxin Structures

Note: Structures are by Fred Fry, Jr., Ph.D.

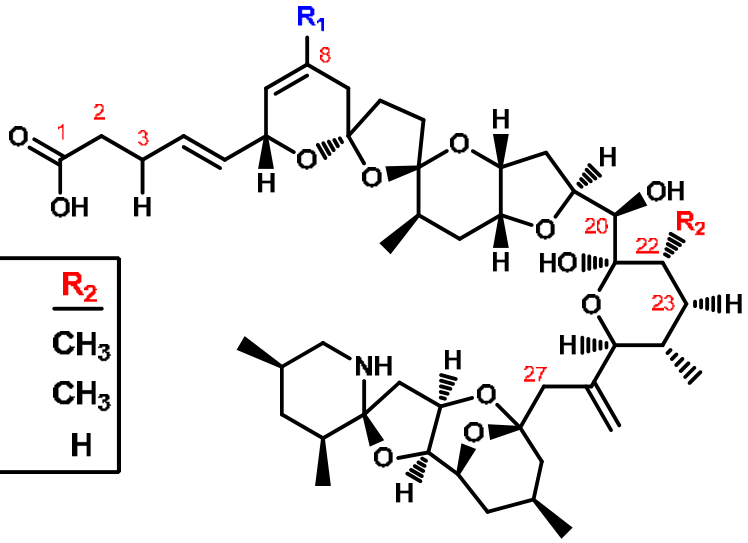
Ciguatoxin (Pacific Ciguatoxin-1 and Caribbean Ciguatoxin-1)

Ciguatoxins are isolated from reef fishes that have ingested and accumulated either these toxins or precursors called gambiertoxins, elaborated by marine dinoflagellates in the genus *Gambierdiscus*.





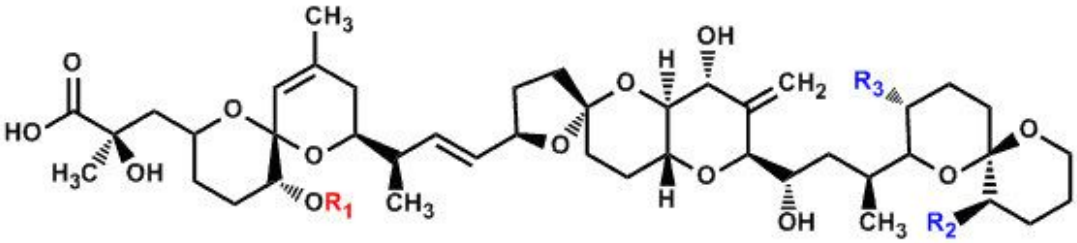
Azaspiracid: AZA analogs produced by the dinoflagellate *Azadinium spinosum* are AZA1, AZA2, and an isomer of AZA2. Major AZA analogs found in shellfish are AZA1, AZA2, and AZA3.



	R_1	R_2
Azaspiracid 1 (AZA1)	H	CH_3
Azaspiracid 2 (AZA2)	CH_3	CH_3
Azaspiracid 3 (AZA3)	H	H

Okadaic Acid and Dinophysis Toxin

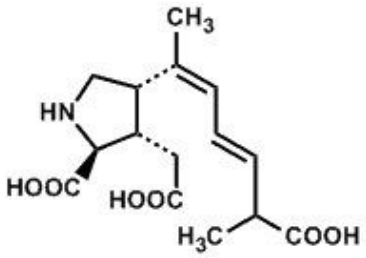
Toxins primarily responsible for DSP are Okadaic Acid (OA), Dinophysistoxin 1 (DTX 1), and Dinophysistoxin 2 (DTX 2). All three of these toxins have a variety of 7-O-acyl ester derivatives that are produced by shellfish and can also cause illness. DSP toxins are produced by select dinoflagellates belonging to the genus *Dinophysis* and *Prorocentrum*.



	R_1	R_2	R_3
OA	H	H	CH_3
DTX1	H	CH_3	CH_3
DTX2	H	CH_3	H
7-O-Acyl	Ac	same as above	

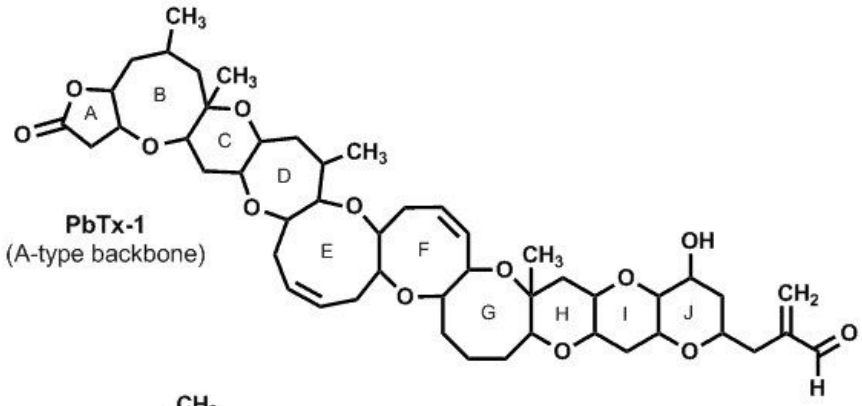
Domoic Acid

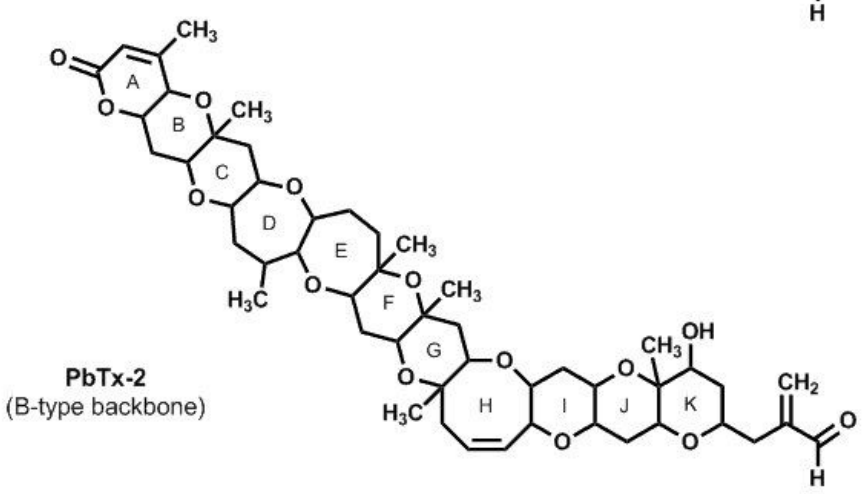
Toxin produced by planktonic algae (certain diatom species) upon which the shellfish feed.



Brevetoxin

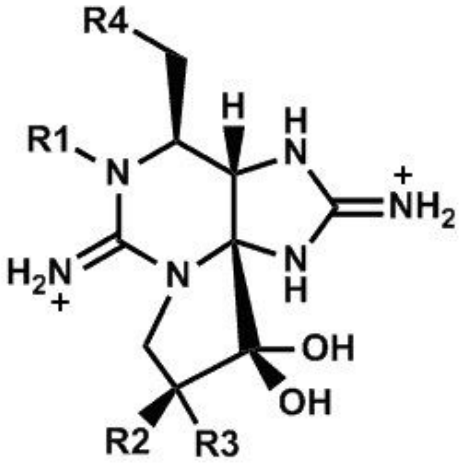
Principal brevetoxins produced by dinoflagellate *Karenia brevis* are PbTx-1 (A-type backbone) and PbTx-2 (B-type backbone). Algal brevetoxins are extensively metabolized in molluscan shellfish. NSP-causing toxins in shellfish include intact algal toxins and their metabolites.

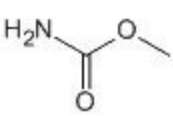
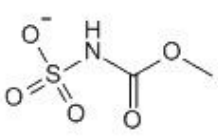
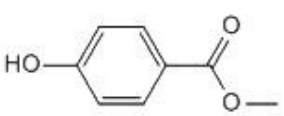




Saxitoxin

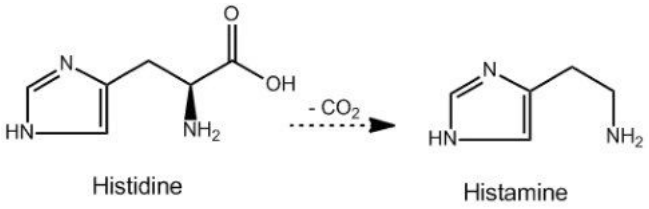
Molecular structure of saxitoxin groups: carbamates (most potent), decarbamoyl toxins (intermediate in toxicity; usually present in shellfish but not toxigenic algae), N-sulfocarbamoyl toxins (less potent), and hydroxybenzoate toxins (more recently recognized group of PSP toxins, shown to be specific to the dinoflagellate *Gymnodinium catenatum*). Toxins in the saxitoxin family may be produced by a range of dinoflagellates, including species in the genera *Alexandrium*, *Gymnodinium*, and *Pyrodinium*. There are also reports of STXs being produced by certain freshwater and brackish cyanobacteria as well as calcareous red macro algae. The traditional route of exposure is accumulation in filter-feeding shellfish.



R1	R2	R3	Carbamate Toxins	Decarbamoyl Toxins	N-sulfocarbamoyl Toxins	Hydroxybenzoate Toxins
H	H	H	STX	dc-STX	B1	GC3
OH	H	H	NEO	dc-NEO	B2	
OH	H	OSO_3^-	GTX 1	dc-GTX 1	C3	
H	H	OSO_3^-	GTX 2	dc-GTX 2	C1	GC1
H	OSO_3^-	H	GTX 3	dc-GTX 3	C2	GC2
OH	OSO_3^-	H	GTX 4	dc-GTX 4	C4	
			R4: 	R4: 	R4: 	R4: 

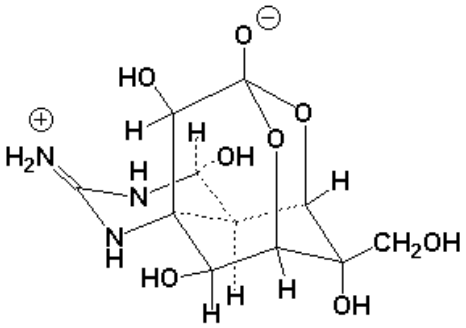
Scombrotoxin: Formation of Histamine from Histidine

Histamine produced by the growth of certain bacteria and the subsequent action of their decarboxylase enzymes on histidine.



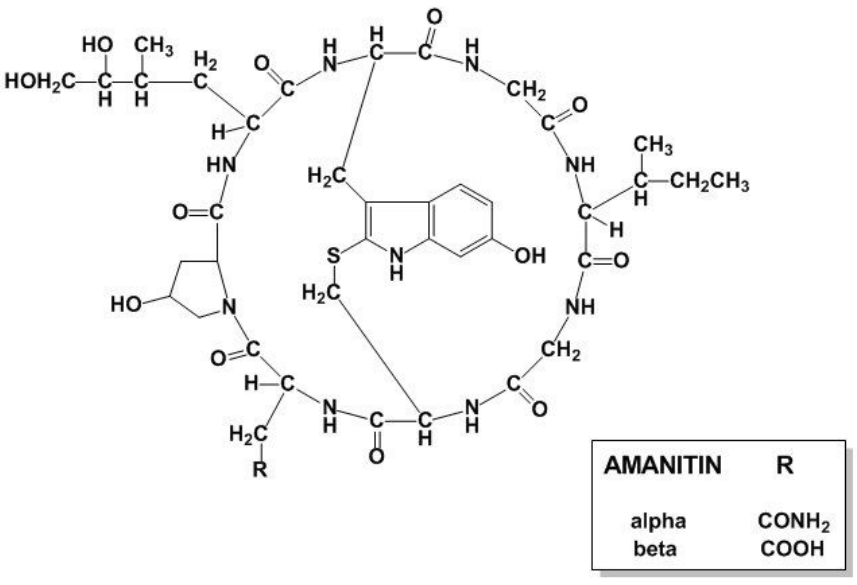
Histidine Histamine

Tetrodotoxin



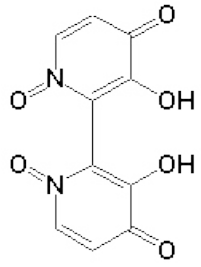
Amanitin

Toxin produced by several mushroom species, including the Death Cap or Destroying Angel (*Amanita phalloides*, *A. virosa*), the Fool's Mushroom (*A. verna*) and several of their relatives, along with the Autumn Skullcap (*Galerina autumnalis*) and some of its relatives.



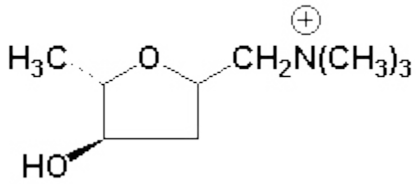
Orellanine

Toxin produced by the Sorrel Webcap mushroom (*Cortinarius orellanus*) and some of its relatives.



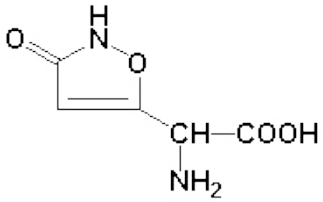
Muscarine

Toxin produced by any number of Inocybe or Clitocybe species (e.g., *Inocybe geophylla*, *Clitocybe dealbata*).



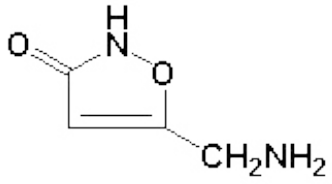
Ibotenic Acid

Toxin produced by Fly Agaric (*Amanita muscaria*) and Panthercap (*Amanita pantherina*) mushrooms.



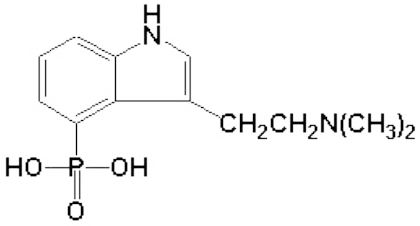
Muscimol

Toxin produced by Fly Agaric (*Amanita muscaria*) and Panthercap (*Amanita pantherina*) mushrooms.



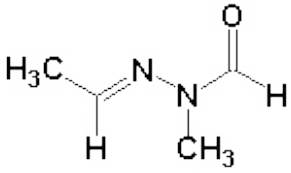
Psilocybin

Toxin produced by a number of mushrooms belonging to the genera *Psilocybe*, *Panaeolus*, *Copelandia*, *Gymnopilus*, *Conocybe*, and *Pluteus*.



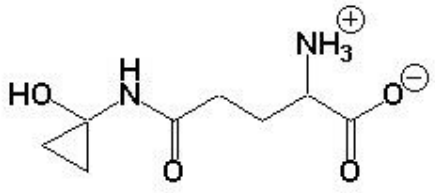
Gyromitrin

Toxin produced by certain species of False Morel (*Gyromitra esculenta* and *G. gigas*).

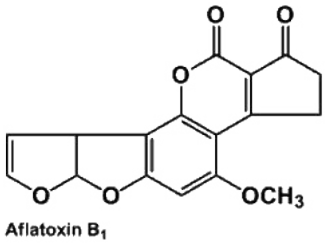


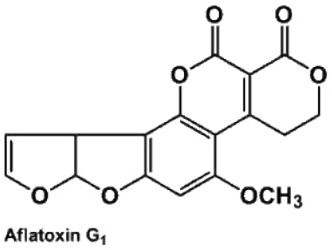
Coprine

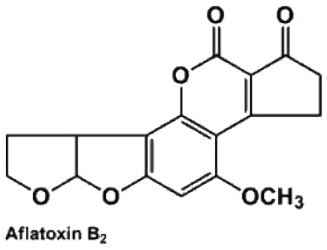
Toxin produced by the Inky Cap Mushroom (*Coprinus atramentarius*).

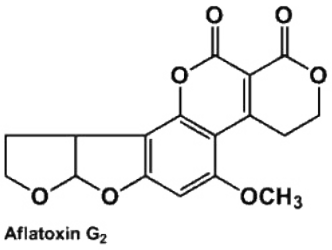


Chemical structures of aflatoxins B_1, B_2, G_1, and G_2

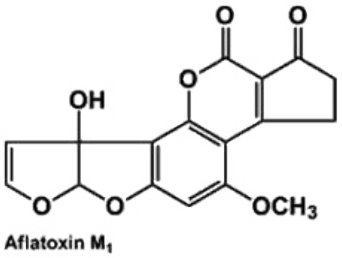






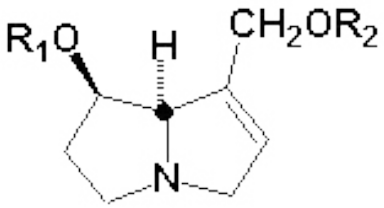


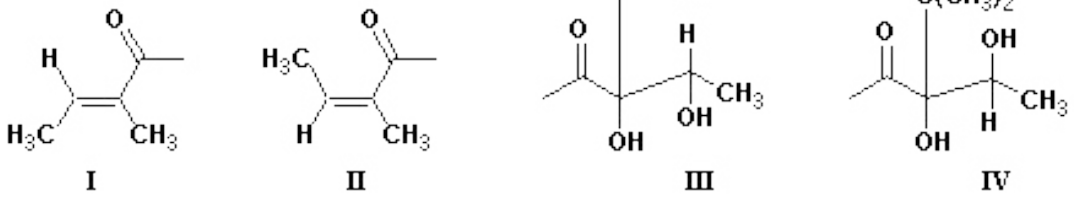
Chemical structure of aflatoxin M_1



Pyrrolizidine Alkaloids of *Symphytum* spp.

Toxins produced by plants from the Boraginaceae, Compositae, and Leguminosae families.

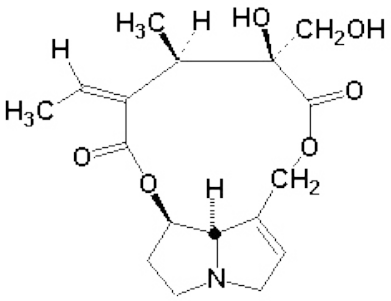




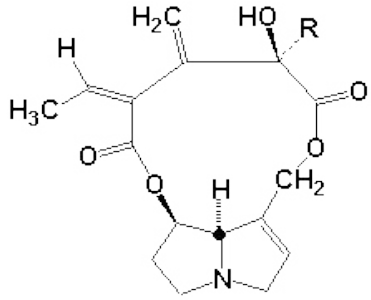
Name	R_1	R_2	R_3
retronecine	H	H	H
lycopsamine	H	III	H
intermedine	H	IV	H
7-acetyllycopsamine	CH_3CO	III	H
7-acetylintermedine	CH_3CO	IV	H
symphytine	I	III	H
symlandine	II	III	H
echimidine	II	III or IV	OH
uplandicine	CH_3CO	III or IV	OH

Pyrrolizidine Alkaloids of *Senecio longilobus* Benth

Toxins produced by plants from the Boraginaceae, Compositae, and Leguminosae families.



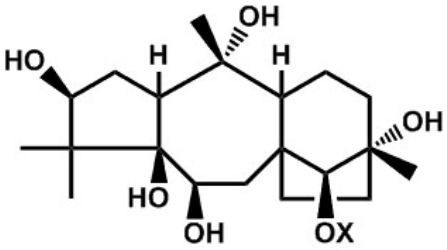
Retrorsine



NAME	R
Seneciphylline	H
Riddelliine	CH_2OH

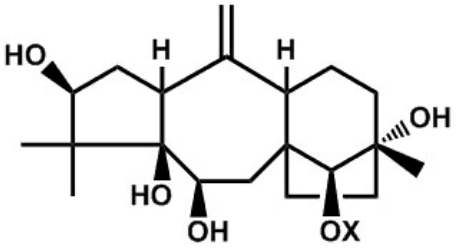
Grayanotoxin

Toxins found in components of some Rhododendron species along with other plants in the Ericaceæ family. Occasionally found in honey derived from these plants.



Grayanotoxin G I (X=Ac)

Grayanotoxin G III (X=H)



Grayanotoxin G II (X=H)

Grayanotoxin G IV (X=AC)

Phytohaemagglutinin

Please refer to the Molecular Structure section of the phytohaemagglutinin chapter of this book.

Technical Glossary

Aerobe – a microorganism that grows in the presence of atmospheric oxygen.

Aflatoxin – a mycotoxin, made by several species of the fungus Aspergillus, that can cause cancer.

Allergy – an immediate immune (hypersensitivity) response to a substance (allergen).

Anaerobe – an organism that grows in the absence of free oxygen.

Antibody – glycoprotein (immunoglobulin) substance developed by the body in response to, and interacting specifically with, an antigen, as part of the body's immune response.

Antigen – a foreign substance that stimulates the formation of antibodies that react with that substance, specifically.

Antisepsis – prevention or inhibition of growth of microorganisms on skin or tissue.

Autoclave – An apparatus for sterilizing objects by use of steam under pressure.

Bacillus / bacilli) – rod-shaped bacterium / bacteria.

Bacteremia – presence of bacteria in the blood.

Bacteria – prokaryotic, microscopic, one-celled microorganisms that exist as free-living organisms or as parasites and multiply by binary fission.

Bacterial colony – a visible group of bacteria growing on a solid medium.

Bactericide – an agent that destroys bacteria, but is not necessarily effective against spores.

Bacteriophage – a virus that infects bacteria; often called a phage.

Binary fission – a method of asexual reproduction involving halving of the nucleus and cytoplasm of the original cell, followed by development of each half into two new individual cells.

Biofilms – organized microbial systems consisting of layers of microbial cells growing on surfaces.

Botulism – a potentially fatal intoxication form of food poisoning caused by a neurotoxin produced by Clostridium botulinum serotypes A-G.

Capsule – the membrane that surrounds and is attached to some bacterial cells; in some pathogenic bacteria, helps to protect against phagocytosis.

Cell wall – In bacterial cells, a layer or structure that lies outside the plasma membrane and provides support and shape to the bacterium.

Colony Forming Unit (CFU) – viable microorganisms (bacteria, yeasts, and mold), capable of growth on solid agar medium, that develop into visible colonies, which can be counted for

diagnostic or research purposes. The colony forming unit may consist of a single cell or a clump of several cells that grow into a single colony.

Coccus / cocci – the type of bacteria that are spherical or ovoid in form.

Colony – a visible population of microorganisms growing on a solid surface of an agar culture medium.

Commensal – a relationship between two organisms, in which one benefits from the other, but the other receives neither benefit nor harm.

Communicable – an infectious disease that may be transmitted directly or indirectly from one host to another.

Contamination – presence of a microorganism or other undesirable material on or in an area or substance (e.g., food) in which it does not belong or is not normally found.

Diplococci – round bacteria (cocci) arranged in pairs.

Disinfectant – a chemical or physical agent used on inanimate surfaces that kills disease-causing bacteria and fungi.

Emetic toxin – a toxin that causes vomiting.

Endemic – a disease that has relatively stable occurrence in a particular region, but has low mortality.

Endospores – a thick-walled spore, formed by certain bacteria, that is resistant to harsh environmental conditions.

Endotoxin – a heat-stable lipopolysaccharide, found in the outer membrane of Gram-negative bacteria, that is released when the bacterium lyses or, sometimes, during growth, and is toxic and potentially fatal to the host.

Enterotoxin – a toxin released from several types of bacteria in the intestine that specifically affect the host intestinal mucosal cells, causing vomiting and diarrhea.

Enteric bacteria – bacterial members of the family *Enterobacteriaceae* that are Gram-negative rods, are nonmotile or motile by peritrichous flagella, and are facultative anaerobes. Commonly used to describe bacteria that reside in the intestinal tract.

Epidemic – infectious disease or condition that affects many people at the same time, in the same geographical area, at a greater-than-normal frequency.

Etiology – the cause of a disease.

Eukaryote – a unicellular or multicellular organism that has a well-defined nucleus and other organelles.

Exotoxin – A usually heat-labile toxin produced by a microorganism and secreted into the surrounding environment.

Facultative anaerobe – a microorganism that is capable of aerobic respiration in the presence of oxygen or fermentation in the absence of oxygen.

Fecal-oral route – a means of spreading pathogenic microorganisms from feces produced by an infected host to another host, usually via the mouth; e.g., contact between contaminated hands or objects and the mouth.

Flagellum / flagella – a long, thin, threadlike structure that extends from many prokaryotic and eukaryotic cells and provides motility.

Fomite – an inanimate object, e.g. utensils, to which infectious material adheres and from which it can be transmitted.

Food intoxication – a form of food poisoning caused by the ingestion of microbial toxins produced in foods prior to consumption. Living microorganisms do not have to be present.

Food poisoning – a term usually indicative of a gastrointestinal illness caused by ingestion of contaminated foods, whether by a pathogen, toxin, or chemical.

Foodborne infection – a form of food poisoning caused by ingestion of foods contaminated with living, pathogenic microorganisms.

Foodborne transmission – spread of pathogenic microorganisms or toxins present in foods that were improperly prepared or stored.

Fungus / fungi – eukaryotic, diverse, widespread unicellular and multicellular organisms that lack chlorophyll, usually bear spores, and may be filamentous. Examples of fungi include yeasts, molds, and mushrooms.

Generation time – the amount of time in which a microorganism doubles in number.

Genome – the total of all genetic material in a microorganism.

Gram-negative cell - a bacterium that has a cell wall composed of a thin peptidoglycan layer, a periplasmic space, and an external lipopolysaccharide membrane. Typical Gram-stain reaction is pink.

Gram-positive cell – a bacterium that has a cell wall composed of a thick layer of peptidoglycan containing teichoic acids. Typical Gram-stain reaction is purple.

Incubation period – time between infection of host with pathogen and appearance of symptoms during an infectious disease process.

Indigenous flora – usually synonymous with “normal flora”; refers to the microbial population that inhabits a host internally or externally.

Infection – the entry, establishment, and multiplication of pathogenic organisms within a host.

Lipopolysaccharide – a polysaccharide found in the cell wall of Gram-negative bacteria that is composed of three components: Lipid A (endotoxin), core, and O-antigen.

Log-phase (exponential) growth – the period during growth of a culture when the population

increases exponentially by a factor of 10.

Maximum temperature – the highest temperature at which a microbe will grow.

Mechanical vector – a living organism that transmits infectious microorganisms from its external body parts or surfaces (rather than excreting the agent from an internal source).

Mesophile – microorganisms that prefer warm growth temperatures, generally between 20^{o}C and 40^{o}C.

Microaerophilic – a microorganism that requires low concentrations of oxygen for growth.

Minimum temperature – the lowest temperature at which a microbe will grow.

Morbidity – disease / illness.

Mortality – the state of being susceptible to death, or the relative frequency of deaths in a specific population.

Mortality rate – ratio of the number of deaths from a given disease to the total number of cases from that disease, per unit time.

Most probable number (MPN) – a statistical means of estimating the size of a microbial population, based on the dilution of a sample, and determining the end points of growth.

Mycotoxins – fungal secondary metabolites toxic to humans and produced by molds.

Optimum temperature – temperature at which microorganisms grow best.

Pandemic – an epidemic occurring at the same time on different continents or a disease affecting the majority of the population of a large region.

Parasite – an organism that benefits from its relationship with its host, at the host's expense.

Pathogen – any microorganism that can cause disease.

Pathogenicity – the ability of a microorganism to produce pathological changes and disease.

Prion – an infectious, misfolded protein that has the capability of causing normal proteins to become misfolded, thereby producing disease. The resulting diseases are called spongiform encephalopathies.

Protozoa – one-celled organisms, existing singly or aggregating into colonies, belonging to a diverse group of eukaryotes that usually are nonphotosynthetic and often are classified further into phyla according to their capacity for, and means of, motility, as by pseudopodia, flagella, or cilia.

Psychrophiles – bacteria with cold optimal growth temperatures, usually between 0^{o}C and 10^{o}C, that do not grow well at mesophilic temperatures.

Psychrotrophs – bacteria that can grow slowly at temperatures below 15^{o}C, but prefer growing at warmer temperatures.

Sauces – Commercial salad dressings, mayonnaise, and acidified sauces are microbiologically safe. Manufacturers follow strict quality controls and diligently comply with FDA-mandated Good Manufacturing Practices in production of these commercial products. Commercial salad dressing, mayonnaise, and sauce products are also made with pasteurized eggs that are free of *Salmonella* and other pathogenic bacteria and further ensure the safety of these products. As such, these commercial products do not have the food-safety risks associated with their homemade counterparts, which contain unpasteurized eggs. Homemade versions also may not contain sufficient quantities of food acids, like vinegar (acetic acid) or lemon juice (citric acid,) to kill harmful microorganisms. As with all foods, the accidental introduction of harmful bacteria from other sources must be avoided, particularly post-manufacture. Consumers should follow sanitary food handling practices in dealing with all foods, including salad dressings, mayonnaise, and sauces, to maintain their safety, and follow manufacturers' directions to keep food refrigerated.

Secondary infection – an infection caused by a different microorganism than the agent that caused a primary infection.

Septicemia – multiplication of bacteria in the blood, potentially leading to sepsis (generalized inflammation of the body).

Spirochete – Gram-negative bacteria having a flexible, helical-shaped cell wall with axial filaments (no flagella) that run the length of the cell and enable it to move by contractions (undulate).

Spore – Bacterial: A thick, resistant cell produced by a bacterium or protist to survive in harsh or unfavorable conditions. Fungal: unicellular or multicellular bodies produced during complex life cycles of fungi that may enhance survival in a hostile environment.

Sterilization – a process that completely eradicates all organisms and/or their products in or on an object.

Strict (obligate) aerobe – a microorganism that will grow and live only in the presence of free oxygen.

Strict (obligate) anaerobe – a microorganism that will grow and live only in the absence of free oxygen.

Strict (obligate) parasite – an organism that is completely dependent on its living host for survival.

Symbiotic – two or more organisms that live in close relationships required by one or both members.

Thermophile – bacteria with relatively high optimal growth temperatures, usually between 40^{o}C and 70^{o}C, that do not grow well at mesophilic temperatures.

Toxin – a poisonous substance produced by microorganisms, plants, or animals. Venoms are toxins injected by animals.

Virulence – the relative ability of a microorganism to produce disease.

Virus – small, non-living, infectious agents, consisting of a protein shell (capsid) and a genome of DNA or RNA (not both), characterized by a lack of independent metabolism and inability to replicate independently; it can replicate only within living host cells. Viruses are classified based on morphology, genome, and whether or not they are encapsulated.

CONSUMER GLOSSARY

Abdomen – the part of the body that contains the stomach and bowels and other organs needed for digesting food, as well as other organs. Examples include the kidneys, spleen, pancreas, gallbladder, and liver. Many kinds of foodborne illness, but not all, cause cramps or pain in the abdominal area.

Amoeba – a type of protozoan. (See definition of "protozoan.")

Antibiotic – a medication that kills bacteria (but not viruses). Most bacteria that can be passed to people through contaminated food don't cause serious illness, in people who are otherwise healthy, and don't require antibiotics. But for some of the more serious illnesses, antibiotics can be life-saving. Different antibiotics kill different bacteria, so using the right kind for each type of foodborne illness is important. That's one reason antibiotics have to be prescribed by a licensed health professional.

Bacteria – Bacteria are made up of one cell. Most bacteria aren't harmful; some are helpful to humans and to the environment. But some can cause illness when they enter the human body, including harmful bacteria that enter with contaminated food or water. Some bacteria make a toxin (see definition) that causes illness. Others cause symptoms not by making a toxin, but by causing a strong reaction by the immune system – the body's way of trying to kill bacteria, viruses, and other substances that don't belong in it.

Bowel – The bowel is much more than just a long "tube" that carries food through the body. It absorbs nutrients and water for the body to use, including minerals ("electrolytes") that are very important regulators of heart, brain, and other organ function. When it works properly, the bowel, with the kidneys and with input from the brain, helps ensure that our bodies contain the right balance of water and electrolytes.

When this balance is off, problems can result, from mild to deadly, depending on how severe the imbalance is. See the definition of "dehydration" and "electrolyte" for information about how diarrhea and vomiting can affect this balance.

Like other organs, the bowel has a blood supply that nourishes it, and mucus that lines it, to help food pass through it. Some kinds of bacteria and worms that cause foodborne illness can cause the bowel to bleed, resulting in bloody diarrhea. Some also cause mucus to be passed with the diarrhea, with or without blood.

The bowel has muscle that tightens up and loosens in waves that keep food moving forward. It happens automatically, without your having to think about it. Disturbances to the bowel, like those from foodborne illness, can cause the muscle to cramp.

Carcinogen – a substance that can cause cancer.

Cell – the smallest life form. Cells contain substances and perform functions that enable the cells to survive and reproduce (make copies of themselves). Bacterial cells and human cells differ from each other in important ways. In human cells, DNA is contained in an inner structure called the nucleus. Bacterial cells contain DNA, but they don't have a nucleus.

Commercial – In this book, "commercial" refers to foods meant for sale, or businesses involved in growing, processing, packaging, storing, distributing, transporting, or selling those products.

Contamination – the presence of bacteria, viruses, worms, parasites, toxins, or other substances that don't belong in food or drinks. Some of these substances cause illness if eaten.

Dehydration – loss of body water, which can be caused by diarrhea, among other things. Diarrhea that's severe or lasts a long time can cause dehydration and serious problems, if it's not treated. It can cause dangerous imbalances between the amount of fluid in the body and certain minerals (electrolytes) that are important for normal function of the heart, brain, and other organs.

In mild cases of diarrhea, drinking fluids can replace the lost water and prevent dehydration. Juices and some sports drinks also can help replace electrolytes. In severe cases, the normal balance of fluid and electrolytes can be restored by I.V. ("intravenously"). Severe dehydration and electrolyte imbalance can be dangerous or deadly, in extreme cases, and needs medical attention.

Developing countries – countries that usually have limited resources, compared with others, and don't have sanitary systems; for example, systems for treating sewage. Water used for drinking isn't the only risk in these countries; another example is that contaminated water might have been used to grow or rinse fruits and vegetables.

DNA – chemical structures that make up genes in humans and other living things, including bacteria, worms, and amoebas, for example. (Viruses are not considered living things). As in humans, their DNA can undergo changes. In some microorganisms, these changes happen very often. As a result, one type of bacterium can include many different versions of itself that have slightly different DNA from each other. The different versions are called different "strains."

The change in DNA can affect the microbe's ability to cause illness in humans, for better or worse; or the severity of the illness; or whether or not an antibiotic that usually works against a bacterium can kill the new strain. There are many types of bacteria and viruses that cause foodborne illness, and the speed with which their DNA can change, repeatedly, is a challenge.

Dysentery – Blood vessels nourish the bowel, and it's also lined with mucus, to help food pass through it. In dysentery, which is caused by some foodborne bacteria and other pathogens, diarrhea usually is severe and contains blood and mucus. Other symptoms are fever and pain in the abdominal area. Dysentery can result in dehydration and electrolyte imbalance. (See definitions of "dehydration" and "electrolytes.")

Electrolytes – minerals that are very important for normal heart, brain, and other organ function. They also help keep the amount of fluid in the body at the right level. Electrolytes are absorbed from food as it passes through the bowel. They enter the bloodstream and travel to the cells of organs, where they are among the substances that enable the organs to function properly.

Diarrhea, particularly if it's severe or lasts a long time, can cause an imbalance between the body's fluid and electrolytes. Repeated vomiting also can cause some electrolyte loss. Depending on the severity of the fluid and electrolyte imbalance, symptoms might include mild to severe weakness, confusion, and irregular heartbeat, among others. In extreme cases, the imbalance can lead to death.

Nausea, vomiting, and cramps, including muscle cramps, also might occur – but those symptoms also can be caused by the foodborne illness itself (by the bacterium or virus, for example), rather than by electrolyte imbalance. If you've had severe or long-lasting diarrhea and have these symptoms, it's important to see a health professional. Laboratory tests can show if these symptoms are from an electrolyte imbalance, and if they are, the amounts of fluids and electrolytes you need to put your body back in balance.

Enterotoxin – a substance that's produced inside some types of foodborne bacterial cells and that causes illness. Some of these kinds of bacteria release the toxin after they're digested in the bowel. Illness from this type can be prevented by cooking the food before it's eaten, which kills the bacteria. But other kinds of bacteria make toxins in the food before it's eaten, and cooking the food doesn't destroy the toxin. When the food is eaten, the toxin is eaten along with it.

Feces – The waste that's passed out of the body after food has gone through the bowel.

Foodborne – carried by food; for example, an illness that was caused by a harmful bacterium in food.

Freshwater – inland water, such as lakes, rivers, streams, and ponds. Some parasites (see definition) that live in freshwater can cause illness in humans if the water is used for drinking or for watering or rinsing fruits and vegetables, for example.

Gastrointestinal – having to do with the stomach and / or bowel.

Genes – see the definition of "DNA."

Hand sanitizer – Sprays, gels, or wipes that can kill many harmful bacterial cells (but not spores – see definition). The alcohol in hand sanitizers doesn't destroy norovirus, the leading cause of foodborne illness in the U.S. Handwashing is the best prevention.

Hygiene – behaviors that prevent disease and help people stay healthy. Examples of hygienic behaviors in this book include handwashing, using clean cooking equipment, and keeping kitchen counters clean.

Immune system – the complex system in the body that attacks bacteria, viruses, and other harmful substance that enter the body. The immune system prevents or stops many infections in this way.

Many chapters in this book caution that people with weak immune systems are more at risk from foodborne bacteria, viruses, and parasites ("pathogens"), compared with people with strong immune systems. They can become infected much more easily, get much sicker, and might not be able to get over the infection. Even foodborne illnesses that are mild, in most people, can be deadly to someone with a weak immune system.

Infection – A bacterium, virus, or other pathogen enters the body and multiplies. The symptoms caused by the infection often are the result of the immune system's response to the pathogen, such as inflammation. (See the definition of "immune system.") Infections may spread out of the site in which they first entered and grow in the body; for example, foodborne pathogens occasionally spread from the bowel into the bloodstream and into other organs.

Intestine – The small and large intestine make up the bowel. See the definition of "bowel."

Minerals – See the definition of "electrolytes."

Mucus – The bowel is lined with mucus, a slippery substance that helps food pass through the bowel. In some foodborne illnesses that cause diarrhea, this mucus is passed with the feces.

Neurologic – having to do with the nervous system (the brain, spinal cord, and nerves). A few types of fish and shellfish sometimes contain toxins that can cause neurologic symptoms. Depending on the toxin and the amount, problems may range from mild light-headedness that goes away by itself to paralysis. Electrolyte imbalance also may cause some neurologic symptoms. (See definition of "electrolytes.")

Outbreak – When two or more people become sick from the same bacterium, virus, or other pathogen, it's called an outbreak. When outbreaks of illness from foods regulated by the Food and Drug Administration (FDA) occur, the FDA, the Centers for Disease Control and Prevention, and state health authorities investigate together, to find the source of the contaminated food that caused the illness, so that the outbreak can be stopped.

Parasite – Certain amoebas and worms that can be passed to humans (and to other animals, in most cases) in contaminated food or water are examples of parasites; once inside humans, they use the human's resources to sustain them, without helping the human in any way. Some make the human sick. Some parasites die naturally in a short time and are passed out of the body. Others, such as tapeworms, can live in the human bowel for years. Most parasites that affect humans are too small to be seen with the naked eye. Worms that affect humans are too small to be seen with the naked eye at the life stage when they can cause an infection, but grow larger inside humans. Water, soil, and hands that are contaminated with feces from an infected person – even particles too small to see – are common ways that parasites are passed into the mouths of humans.

Pasteurization – a process used on some foods and drinks, by food manufacturers, to kill the kinds and amounts of bacteria that can cause illness. Pasteurization applies a certain amount of heat for a certain amount of time, depending on the type of food or drink and the bacteria that are able to live and grow in it. Pasteurization isn't appropriate for some foods. And even though a food may be pasteurized, it still has to be stored properly afterwards; otherwise, harmful bacteria could grow in it.

Milk is one example of how pasteurization helps keep foods safe. ***Un***pasteurized ("raw") milk and certain cheeses made from raw milk can contain harmful amounts of bacteria, such as the types of *E. coli*, *Listeria*, and *Brucella* that cause illness. Even though ***un***pasteurized milk has caused many illnesses and even has resulted in deaths, some people claim that it's healthier than pasteurized milk. There's no scientific evidence to support this.

Pathogen – a life form, such as a bacterium or protozoan (see definition), that can cause disease. Viruses are not life forms, but some cause disease and are among the pathogens.

Poison – chemical substances that can sicken living things. Some poisons have only mild effects, but some can be deadly. **Toxins** are poisons made by living things, such as the enterotoxins (see definition) made by some kinds of bacteria. **Venoms** are poisons that some animals, such as snakes, wasps, and lionfish, inject into other living things. Cooking, freezing, and other kinds of food preparation don't destroy the toxins made by some bacteria – but cooking can kill the bacteria themselves, in most cases.

Protozoan – a life form made of a single cell that lives in water or soil and is able to move on its own. One of the ways they differ from bacteria is that protozoan cells have a nucleus, which contains their DNA. Protozoans can act as parasites (definition appears above) and cause illness in humans. When they're still developing – in the cyst stage of their lives, for example – some may contaminate food or water and, if eaten, develop fully inside a human or animal and cause symptoms. They produce more cysts, which then are passed through bowel movements into the outside world. There, the cysts can withstand harsh conditions – some can even withstand chlorine – and be picked up again, by somebody else, through contaminated food or water, such as water for drinking, recreation, or crop irrigation or rinsing. Another way protozoans spread is by person-to-person contact; for example, by infected people who don't wash their hands well after a bowel movement or after cleaning an infected person who has had a bowel movement.

Raw milk – milk that hasn't been pasteurized. Some of the more dangerous kinds of foodborne bacteria may be present in raw milk; for example, the types of *E. coli* that cause illness; *Listeria monocytogenes*; and *Brucella*. See the definition of "pasteurization" for more information.

Refrigeration – It takes a certain number of cells of a bacterium to cause illness. For a few types of bacteria, the number is low, but, for many types, a fairly high number of bacterial cells has to be present in food to cause illness. That's one reason refrigeration is so important to food safety. If food is kept at 40ºF or below, it keeps bacterial cells from multiplying in food or greatly slows down the growth (with just a few exceptions). Refrigerating food quickly after it's cooked also is important.

As important as refrigeration is, there are good reasons ***not*** to count on it as your only food-safety measure. As noted, a few bacteria can multiply at refrigeration temperatures and even at average home-freezer temperatures. And unlike bacteria, which thrive on warmth, norovirus is most stable at cool storage temperatures. Follow *all* of the basic food safety tips to protect yourself.

Reported illness – Health professionals are required to report cases of some kinds of illness to state health authorities, to help them understand what kinds of illness are in the community and prevent them. The states report the cases to the Centers for Disease Control and Prevention (CDC). The CDC uses this information to track patterns of illness in the U.S., which helps to show what kinds of prevention efforts are needed, and where. Because not everyone who is sick sees a health professional, some cases of illness go unreported. When the chapters of this book refer to "reported illnesses," it means only the cases in which someone saw a health professional. The numbers of cases probably would be substantially higher if unreported cases could be included.

Sanitary – conditions and behaviors that help prevent disease; for example, sanitary water is clean and free of bacteria, viruses, protozoans, and other substances that can make people sick. An example of a sanitary practice in the home is keeping cooking areas clean.

Spore (endospore) – A few bacteria, including some that can cause foodborne illness, can produce inactive forms called endospores. The bacteria do this when their survival is threatened; for example, when there is very little or no nutrition available to them. Endospores can exist for many years and in very tough conditions. They don't need nutrition and can withstand heat, freezing, and disinfectants. When conditions improve, the spores become active bacteria again. Like bacteria, endospores can contaminate food and water.

Stool – Another word for "feces," defined above.

Toxin – a natural poison made by a living thing; for example the toxins made by some bacteria.

Venom – a natural poison that some animals make and inject into others through a "sting."

Virus –Viruses aren't living things; they are basically just DNA, or the similar substance RNA, covered by protein (and fat – lipids – in some cases). Unlike bacteria, they don't have the substances needed to reproduce themselves. Instead, a virus enters the cells of other living things, including humans, and uses the substances in those cells to reproduce itself. The virus can make hundreds to thousands of copies of itself in this way.