Update on *Salmonella* Foodborne Gastroenteritis
(Revised 8/7/14)

Course # DL-003

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**COURSE NAME**  UPDATE ON *SALMONELLA* FOODBORNE GASTROENTERITIS  
**COURSE #**  DL-003

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UPDATE ON SALMONELLA FOODBORNE GASTROENTERITIS

Course #DL-003
3.0 CE
Level of Difficulty: Intermediate

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OUTLINE
A. Introduction
B. History of Salmonella Gastroenteritis
C. Salmonella Nomenclature
D. Transmission of Salmonella
E. Illness/Symptoms of Salmonella Gastroenteritis
F. Complications of Salmonella Gastroenteritis
G. Microbiology of Salmonella
H. Pathogenic Mechanisms of Salmonella
I. Diagnosis and Identification of Salmonella Gastroenteritis
J. Treatment of Salmonella Gastroenteritis
K. How to Prevent Salmonella Gastroenteritis
L. Conclusions
M. References

COURSE OBJECTIVES
After completing this course the participant will be able to:
- outline the history of Salmonella gastroenteritis
- discuss the incidence of Salmonella infection in the U.S.
- explain the pathogenicity factors of Salmonella
- describe the symptoms of Salmonella gastroenteritis
- explain how Salmonella is identified
- state methods to prevent Salmonella gastroenteritis
- outline methods of treatment of Salmonella

A. INTRODUCTION
Foodborne infections are an important public health problem in the United States (1). For the year 2010, the Centers for Disease Control and Prevention (CDC) estimated that all foodborne infections from various viral and bacterial sources caused 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the U.S. (1,2). Most foodborne infections are self-limiting and the symptoms usually go away in 5-7 days; however, in some instances there can be serious complications.

Salmonella gastroenteritis, also called salmonellosis, is the most common bacterial foodborne infection in the United States. Every year in the U.S, approximately 40,000 cases of Salmonella gastroenteritis infections are reported to the CDC, and the incidence of Salmonella
gastroenteritis over the last 10 years has been increasing (1,2). See Figure 1. Number of Foodborne Infections by Year. In 2013, there were 4,563 hospitalizations and 68 deaths due to Salmonella foodborne infection reported to the CDC (9). It is estimated, however, that only 3% of all Salmonella cases are laboratory confirmed and reported to the CDC. Many milder cases are not diagnosed or reported, so it is believed that the actual incidence is 1.4 to 4 million Salmonella infections each year with approximately 400 deaths (2).

Foodborne Salmonella gastroenteritis can affect all ages, but the incidence, severity of disease, and potential complications of the infection are higher in young children, the elderly, and people who are immunocompromised or have debilitating diseases. Children are particularly susceptible to Salmonella foodborne infection. The rate of diagnosed infections in children less than five years old is about five times higher than the rate in the rest of the population (3).

Most persons infected with Salmonella develop diarrhea, nausea, mild fever, and abdominal cramps 8 to 72 hours after infection. Usually the symptoms resolve on their own without medical treatment in 5-7 days, although it may be several months before bowel habits are entirely normal. In some persons, however, the diarrhea and fluid loss may be so severe that the patient needs to be hospitalized and requires rehydration with intravenous fluids. Antibiotics are not recommended unless the infection spreads outside the intestines. Rarely, the organism invades the patient’s bloodstream (bacteremia). In about 5-10% of patients who develop Salmonella in their bloodstream, secondary extraintestinal infections may occur in the endocardium, lungs, bones, joints, kidneys, and soft tissues (4). Some patients may develop Reiter’s syndrome, another complication due to Salmonella gastroenteritis that results in joint pain, eye irritation, and painful urination. See Section F. Complications of Salmonella Gastroenteritis.

The genus Salmonella is named after Dr. Daniel Salmon, an American veterinary pathologist who studied diarrhea in animals, particularly hogs. In 1885, his studies led to the identification of the causative organism that subsequently was named Salmonella cholera-suis (4). The USDA named the genus after him for his contributions to veterinary science. Studies by other investigators led to a greater understanding of the source, transmission, and diagnosis of Salmonella infection.

Salmonella live as part of the normal intestinal flora of both cold and warm blooded animals, such as beef, pigs, poultry, birds, and reptiles (3). The bacteria can be found throughout the natural environment, including soil and water that has been contaminated with animal feces. Although the bacteria cannot multiply outside of the host digestive tract, they can live for several weeks in water and several years in soil when conditions such as humidity, pH, and temperature are favorable (3,4).

Salmonella are transmitted to humans when people eat food or drink water that is contaminated with animal feces. Contaminated foods are often of animal origin, such as beef, poultry, milk, or eggs, but any food, including vegetables, may become contaminated. Food may also become contaminated by the hands of infected food handlers who do not wash hands with soap and water after using the bathroom. Salmonella has also been found in the feces of some pets (dogs, cats, birds, and reptiles), and people can become infected if they do not wash their hands after contact with pets or pet feces (3). See Section D. Transmission of Salmonella.

Salmonella Typhi, while found in the water supply in some parts of the world, is not typically associated with food-borne infection in the U.S. (1). Food in other parts of the world can be contaminated with S. Typhi if is it washed or irrigated with contaminated water, producing typhoid fever. This disease is very rare in the U.S. and is typically acquired today by
people visiting an underdeveloped country. Therefore, S. Typhi will not be described in this course.

This Distance Learning Course will review some of the history of foodborne infections due to *Salmonella* and will discuss where the organism is normally found, how the organism is spread, the clinical symptoms of the disease, complications of the disease, how the organism is isolated and identified by the clinical laboratory, the virulence mechanisms of *Salmonella*, and some steps people can take to reduce the risk of infection.

**B. HISTORY OF SALMONELLA GASTROENTERITIS**

The symptoms of foodborne *Salmonella* gastroenteritis have been described throughout much of recorded history, although the responsible agent and source of the infection were not understood until the 19th century. Some of the earliest reports of food-borne infection due to *Salmonella* date back to at least 323 B.C. and Alexander the Great (4). While the actual cause of his illness is not known, reports of his diarrhea and other symptoms, and the symptoms of his soldiers, are generally associated with foodborne *Salmonella* gastroenteritis. Subsequent foodborne outbreaks have been reported throughout history, particularly during times of stress, crowding, wars, and famines.

The causative organism of foodborne *Salmonella* infection was first identified in 1885 when Dr. Salmon isolated *Salmonella cholera-suis* from the intestines of pigs with symptoms of severe diarrhea that resembled cholera (4). It was later found that *Salmonella cholera-suis*, as well as other species of *Salmonella*, were associated with human foodborne diarrheal disease. However, the large foodborne outbreaks from wide-spread geographical areas that we experience today did not exist until relatively recently. Up until the 1930s, most food consumed was obtained from nearby farms and produced in small amounts. If some of the food was contaminated with *Salmonella*, it made only a small number of people sick, all of whom likely resided within the immediate area of where the food was produced. There have been enormous changes in food-production practices since the 1930s, some of which have probably increased our risk and exposure to *Salmonella*-contaminated food. Today, food processing is accomplished on an industrial scale, and food is distributed to a very large geographic area. Food can be distributed over the entire United States in a short period of time and can remain available for consumers for months if the food has a long shelf life. Therefore, food contaminated with *Salmonella* today can cause wide-spread outbreaks affecting many people from many states.

In 1995, the Foodborne Diseases Active Surveillance Network, or FoodNet, was created to collect and monitor more precise information on the incidence of foodborne disease in the United States. FoodNet has links with 10 state and local health departments at selected sites nationwide, representing 15% of the U.S. population. These health departments collect data on laboratory-confirmed infections from nine pathogens transmitted via food (5). The surveillance performed by FoodNet attempts to obtain the most complete and current “real-time” data on foodborne illness so that outbreaks can be investigated promptly and contained. Although the FoodNet surveillance system provides the best available estimate of the incidence of various pathogens transmitted through food, there are a number of limitations to the system, and the data do not reflect the true nation-wide incidence of infection because many foodborne illnesses are not laboratory-confirmed nor reported to FoodNet.

The data from FoodNet shows that *Salmonella* is the most common foodborne bacterial pathogen in the United States (2,5, 9). There were 7,800 cases of *Salmonella* and 6,793 cases of *Campylobacter* reported to FoodNet in 2013. See Table 1, Number of Laboratory-Confirmed
Infections by State in 2019 (9). *Salmonella* infection accounts for almost 40 percent of all foodborne bacterial illness each year and is associated with the greatest number of hospitalizations (4,563) and deaths (68) in 2013, causing an estimated $365 million in direct medical cost due to this organism alone (2,9). The incidence of reported *Salmonella* gastroenteritis cases to FoodNet has increased from 6,351 in 2004 to 7,800 in 2013 (9). See Fig. 1, Number of Foodborne Infections by Year. In some states (e.g. Georgia, Maryland, Oregon, and Tennessee), salmonellosis is the most commonly reported cause of gastroenteritis, while in others it is the second most common compared to *Campylobacter*. See Table 1, Number of Laboratory-Confirmed Infections by State in 2013. Most of the *Salmonella* serotypes that cause disease in humans in the United States belong to *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Salmonella* Newport. These three serotypes of *Salmonella* account for about half of culture-confirmed *Salmonella* isolates reported to the CDC (1,2).

**Recent Salmonella Outbreaks in the United States.** While there are many sporadic cases of *Salmonella* infection, the following examples from 2010 and from 2011 of relatively large foodborne *Salmonella* outbreaks reported to the CDC will give the reader a perspective of the incidence and common source of infection (6).

In 2010, there were 12 large outbreaks from multiple states reported to the CDC (6). The sources and number of cases from some of the outbreaks included alfalfa sprouts (140 cases from 26 states), cheese (75 cases from 15 states), chicken rice frozen entrée (44 cases from 18 states), fruit pulp (50 cases from 7 states), frozen rodents (34 cases from 17 states), romaine lettuce (60 cases from 12 states), red and black peppers (272 cases from 44 states), water frogs (85 cases from 31 states), and beef and poultry (119 cases from 17 states). One of the largest outbreaks in 2010 was due to *Salmonella* Enteritidis, involving 1,939 individuals from 11 states and caused by contaminated shell eggs from a supplier in Iowa providing eggs to 29 restaurants (6). Other *Salmonella* outbreaks in 2010 were related to pet birds, dogs, cats, ground beef, cantaloupe, unpasteurized milk, unpasteurized ice cream, pet treats or pet food, tomatoes, contaminated spices from China, unpasteurized Mexican-style cheese, hummus, peanut butter, pot pies, contaminated puffed rice or wheat cereals, and raw jalapeno and serrano peppers (6).

In 2011, there were 16 large outbreaks from multiple states reported to the CDC (6). The sources and number of cases from some of the outbreaks included Turkish pine nuts (43 cases from 15 states), ground beef (119 cases from 7 states), romaine lettuce (87 cases from 12 states), chicken livers (34 cases from 6 states), cantaloupe (200 cases from 12 states), ground turkey (136 cases from 39 states), papayas (106 cases from 26 states), African dwarf frogs (241 cases from 42 states), alfalfa sprouts (125 cases from 5 states), hazelnuts (122 cases from 17 states), and turkey burgers (141 cases from 10 states).

One of the more unusual *Salmonella* outbreaks in 2011 was from a strain of *S. Typhimurium* used in clinical and teaching microbiology laboratories. Because the outbreak was particularly relevant to the clinical laboratory, the CDC’s investigation and advice to teaching and clinical laboratories is included here:


“CDC is collaborating with public health officials in many states to investigate a multistate outbreak of *Salmonella* Typhimurium infections associated with exposure to clinical and teaching microbiology laboratories. Investigators are using DNA analysis of *Salmonella*
bacteria obtained through diagnostic testing to identify cases of illness that may be part of this outbreak.

“As of April 20, 2011, a total of 73 individuals infected with the outbreak strain of *Salmonella* Typhimurium has been reported from 35 states.

“In an epidemiologic study conducted during February and March 2011, 32 ill persons answered questions about exposures during the days before becoming ill. Investigators compared their responses to those of 64 persons of similar age previously reported to state health departments with other illnesses (controls). Preliminary analysis of this study has suggested exposure to clinical and teaching microbiology laboratories is a possible source of illness. Illnesses have been identified among students in microbiology teaching laboratories and employees in clinical microbiology laboratories. Ill persons (60%) were significantly more likely than control persons (2%) to report exposure to a microbiology laboratory in the week before the illness began. Additionally, multiple ill persons reported working specifically with *Salmonella* bacteria in microbiology laboratories. The New Mexico Department of Health found that the outbreak strain was indistinguishable from a commercially available *Salmonella* Typhimurium strain used in laboratory settings. This commercially available strain was known to be present in several teaching or clinical laboratories associated with ill students or employees infected with the outbreak strain. These data suggest this strain is the source of some of these illnesses. Additionally, several children who live in households with a person who works or studies in a microbiology laboratory have become ill with the outbreak strain.

“As part of this ongoing investigation, CDC is working with state and local health departments, the American Society for Microbiology, and the Association of Public Health Laboratories to conduct a survey of laboratory directors, managers, and faculty involved with clinical and teaching microbiology laboratories to identify areas where improvements in biosafety and laboratory safety training can be made to prevent future illnesses.

“Advice to Students and Employees in Clinical and Teaching Microbiology Laboratories:

1. Be aware that bacteria used in microbiology laboratories can make you or others who live in your household sick, especially young children, even if they have never visited the laboratory. It is possible for bacteria to be brought into the home through contaminated lab coats, pens, notebooks and other items that are used in the microbiology laboratory.

2. Persons working with infectious agents, including *Salmonella* bacteria, must be aware of potential hazards, and must be trained and proficient in biosafety practices and techniques required for handling such agents safely, including:

   • Wash hands frequently while working in and immediately after leaving the microbiology laboratory and follow proper hand washing practices. This is especially important to do before preparing food or baby bottles, before eating, and before contact with young children.

   • Do not bring food, drinks, or personal items like car keys, cell phones, and mp3 players into the laboratory. These items may become contaminated if you touch them while working or if you place them on work surfaces.

   • Do not bring pens, notebooks, and other items used inside of the microbiology laboratory into your home.

   • Wear a lab coat or other protective uniform over personal clothing when working in a microbiology laboratory; leave it in the laboratory when you are finished. Remove protective clothing before leaving for non-laboratory areas
(e.g., cafeteria, library, or administrative offices). Dispose of protective clothing appropriately or deposit it for laundering by the institution.

3. If you work with Salmonella bacteria in a microbiology laboratory, watch for symptoms of Salmonella infection, such as diarrhea, fever and abdominal cramps.

“Advice to Laboratory Directors, Managers, and Faculty Involved with Clinical and Teaching Microbiology Laboratories:

1. A comprehensive set of biosafety guidelines for work with Salmonella and other similar human pathogens can be found in the Biosafety Level 2 section of the CDC/NIH Biosafety in the Microbiological and Biomedical Laboratories manual and the Guidelines for Biosafety Laboratory Competency, MMWR Supplement.

2. Non-pathogenic (attenuated) bacteria strains should be used when possible, especially in teaching laboratories. This will help reduce the risk of students and/or their family members becoming ill.

3. Persons working with infectious agents, including Salmonella bacteria, must be aware of potential hazards and be trained and proficient in the practices and techniques required for handling such agents safely.

4. Advise persons using the laboratory to watch for symptoms of Salmonella infection, such as diarrhea, fever, and abdominal cramps, and to call their health care provider if they or a family member have any of these symptoms.

5. All students and employees using the laboratory should be trained in biosafety practices.

6. Ensure that handwashing sinks have soap and paper towels. Require students and employees to wash their hands before leaving the laboratory.

7. Do not allow lab coats to leave the microbiology laboratory, except to be cleaned.

8. Do not allow food, drinks, or personal items to be used while working in the laboratory or placed on laboratory work surfaces.

9. Place dedicated writing utensils, paper, and other supplies at each laboratory station. These items should not be allowed to leave the laboratory.”

Other minor outbreaks in 2011 were related to exposure to pet birds, pet rodents, dogs and cats, chicks and ducklings, shell eggs, unpasteurized milk, unpasteurized ice cream, contaminated dry dog food, fruit salad served at a healthcare facility, Lebanon bologna, unpasteurized Mexican-style cheese, hummus, and peanut butter (6).

In 2012 there were 10 large outbreaks from multiple states reported to the CDC (6,9). The sources and number of cases from some of the outbreaks included peanut butter (42 cases from 20 states), mangoes (127 cases from 15 states), cantaloupe (261 cases from 24 states), ground beef (46 cases from 9 states), dry dog food (49 cases from 20 states), poultry (46 cases from 11 states), pet turtles (473 cases from 43 states), tuna products (425 cases from 28 states), and one outbreak from a very unusual source, pet hedgehogs (26 cases from 12 states). In 2013 there were 8 large outbreaks from multiple states reported to the CDC (6,9). The sources and number of cases included Foster Farms Chicken (524 cases from 25 states), Tahini Sesame Paste (16 cases from 9 states), live poultry (158 cases from 30 states), cucumbers (84 cases from 18 states), chicken meat (134 cases from 13 states), ground beef (22 cases from 6 states), and pet turtles (473 cases from 43 states).

C. SALMONELLA NOMENCLATURE
There is a great deal of confusion over the nomenclature of *Salmonella* strains, and even those who study *Salmonella* are confused at times (4). At best, the terminology given to the species of *Salmonella* is complex and still evolving as more molecular techniques are employed.

The original nomenclature of the genus *Salmonella* was based on species names given according to the host animal from which the strain was first isolated, e.g., *Salmonella typhimurium* (mouse typhoid fever), *Salmonella cholera-suis* (hog cholera), *Salmonella abortus-ovis* (abortion in sheep), and so on. After it was recognized that host specificity did not exist for *Salmonella*, newly isolated strains received species names according to the geographical location from which the new strain was isolated, e.g., *S. london*, *S. panama*, *S. stanleyville*, etc. Later, food sources of the *Salmonella* infection were used to provide species names. One of my favorites is *Salmonella banana*, which described the source of the infection from bananas.

In 1946, two microbiologists, Drs. Kauffman and White, wanted to improve the classification of the genus *Salmonella* based on surface antigens to provide serotypes or serovars (short for serological variants) for the genus. This method is called the Kauffman and White scheme. They found that *Salmonella* could be characterized according to their O and H antigen types. The O antigen type is determined based on the polysaccharide content of the cell wall. The H antigen type is determined based on flagellar proteins called flagellins. In addition, pathogenic strains of *Salmonella Typhi* carry the “Vi” antigen (Vi for virulence), which is part of the bacterial capsule. Each O antigen was given an alpha-numeric determination, e.g., A, B, C1, C2, D, F1, E1, etc., in addition to a species name previously given to the organism. For example, *Salmonella* Typhimurium may have a variety of serotypes of group “B” O antigens, and it may also have a variety of serotypes of H antigens (described as phase 1 and phase 2 flagellar antigens). Each antigenic variation of O and H antigens was considered to be a separate species (if this concept were used today, it would result in over 2,500 species of *Salmonella* because there are currently 2,500 serotypes of *Salmonella*). When *Salmonella* are designated by their antigenic formula using the Kauffman and White classification scheme, the species name is written in Roman letters (not italicized) and begins with a capital letter followed by its antigenic formulae. The antigenic formula would be listed in this format: species [space] O antigens [colon] phase 1 H flagellar antigens [colon] phase 2 H flagellar antigens if present. For example, one serovar of *Salmonella* Typhimurium might be listed as *Salmonella* Typhimurium serotype II 39:z10: z6. One serovar of *Salmonella* Enteritidis might be written as *Salmonella* Enteritidis serotype I 9:12:gm4.

The Kauffman and White scheme was replaced by another development in *Salmonella* nomenclature that occurred in 1973 when it was shown by DNA hybridization techniques that all serotypes of *Salmonella* and all serotypes of “Arizona” (which used to be its own genus) were related at the species level; thus, they belonged to a single species. The only exception is *S. bongori*, which by DNA hybridization is a distinct species. Currently, there are two recognized species of *Salmonella*: *S. enterica* and *S. bongori*. Further, *S. enterica* is divided into 6 subspecies: *S. enterica* subsp. *enterica*, subspecies I; *S. enterica* subsp. *salamae*, subspecies II; *S. enterica* subsp. *arizonae*, subspecies IIIa; *S. enterica* subsp. *diarizonae*, subspecies IIIb; *S. enterica* subsp. *houtenae*, subspecies IV; and *S. enterica* subsp. *indica*, subspecies V. Subspecies IIIa and IIIb represent organisms originally described in the genus “Arizona.” Subspecies I strains are commonly isolated from humans and warm-blooded animals. Subspecies II, IIIa, IIIb, IV, and VI strains are usually isolated from cold-blooded animals and the environment, but still can cause disease in man. Subspecies V has been given to *S. bongori*.
The nomenclature of *Salmonella* has changed substantially in recent years. The terms “serotype” and “serovar” are both frequently used, but the term serovar is preferred to the term serotype. To avoid confusion between serotypes and species, the serotype name is not italicized and starts with a capital letter. For example, the common strain of *S. typhimurium* is now technically known as *Salmonella enterica* serovar Typhimurium. This can be shortened to *Salmonella* Typhimurium. However, since this nomenclature is not in agreement with the traditional nomenclature familiar to most personnel in microbiology and specialists in infectious disease, you will see the organism commonly listed as *Salmonella typhimurium*.

**D. TRANSMISSION OF SALMONELLA**

The primary mode of transmission for foodborne *Salmonella* infection is the fecal-oral route, meaning that a person becomes infected by ingesting food or water that has been contaminated with fecal matter containing the bacteria. People can also obtain *Salmonella* gastroenteritis by directly ingesting contaminated animal feces. *Salmonella* infection, or salmonellosis, is therefore considered a zoonotic infection (an infection caused by an organism that is part of the normal flora in some animals, but causes disease when transmitted to humans). Ninety-five percent of cases of *Salmonella* infection are from contaminated food and water, and 5% of *Salmonella* cases are from direct exposure with contaminated animal feces (1,2,3). The highest incidence of *Salmonella* infection occurs during the months of May through October in most climates.

Historically, the major food sources most likely to cause foodborne *Salmonella* infection are contaminated, inadequately cooked foods of animal origin, such as poultry, beef (including ground beef), eggs, and unpasteurized dairy products. Data from the CDC shows that poultry accounts for 29% of *Salmonella* foodborne infections, eggs account for 18% of infections, vegetables (including sprouts, leafy greens, root vegetables, grains, beans, fruits, and nuts) account for 13% of infections, pork accounts for 12% of infections, and beef accounts for 8% of infections. (3,5,6). While poultry, eggs, vegetables, pork and beef are the most common sources of outbreaks, other food vehicles such as unpasteurized orange juice, raw bean sprouts, mayonnaise, shellfish, and peanuts account for 20% of infections (3,5,6). These foods can become contaminated at almost any step between farm and table and ingested, especially if improperly prepared. Food may also become contaminated by the hands of infected food handlers who do not wash their hands with soap and water after using the bathroom. Workers who have been ill can shed *Salmonella* for a median of 30 days (range, 2 days to 280 days). See Table 2, Risk Factors for *Salmonella* Infection.

*Salmonella* outbreaks have been reported after the ingestion of clean, intact, grade A eggs (7). Not only can the exterior of eggs be potentially contaminated with *Salmonella* from chicken feces, but it has been shown that *Salmonella* can infect the ovaries of healthy-appearing hens and contaminate egg contents before shells are formed (7). *Salmonella* can be inside normal appearing eggs and, if the eggs are eaten raw or undercooked, the organism can cause illness. Studies show that an infected hen can lay many normal eggs and only occasionally lay an egg contaminated with *Salmonella* before returning to lay normal, uninfected eggs (7). *Salmonella* can infect flocks of chickens without causing visible disease and then spread rapidly from hen to hen. When tens or hundreds of thousands of chickens live together, die together, and are processed together, a *Salmonella* infection can rapidly spread throughout the whole food chain. Overcrowding and inattention to good hygiene contribute to the *Salmonella* colonization of the flocks. A factor in the spread of *Salmonella* foodborne illness is that chickens or eggs from a single farm may be
distributed over many cities and many states, so the infection can be rapidly dispersed to many people.

Over the past 10 to 15 years, an increase of *Salmonella* infections has been associated with consumption of fresh fruits and vegetables, including seeds, sprouts, spices, and herbs (6). Fruits and vegetables can become contaminated with animal feaces during growth, transport, or processing. The bacteria can grow into the plant from the roots, as with leafy plants such as spinach, greens, or lettuce, or bacteria can grow within or on other plants such as tomatoes (6). Large, geographically dispersed outbreaks associated with environmental contamination of produce have recently been seen, such as the large multi-state outbreaks of *Salmonella* infections associated with raw tomatoes in 2010 and 2011 involving hundreds of people (6).

A wide variety of animals serve as reservoirs for *Salmonella*, such as cattle, sheep, poultry (including chicks and ducklings), pigs, reptiles, amphibians, birds, dogs, cats, puppies and kittens, and cage birds. Many of these animals shed *Salmonella* continuously or intermittently in feces. Other reservoirs of *Salmonella* are rodents and pets, such as iguanas, tortoises, lizards, turtles, frogs, and snakes. See Table 3, Animal Sources of *Salmonella* Infection. As many as 90% of reptiles may be carriers of *Salmonella* and should always be considered a potential source of infection since they may shed the organism continuously (3,6). Small pet turtles were very popular in the United States during the 1950s and 1960s, until it was discovered that turtles were infected with *Salmonella* and young children were acquiring the organism. These turtles are banned in most states today. Some dogs and cats can shed *Salmonella* spp. for up to three months after they are infected (6).

*Salmonella* can survive for long periods in the environment, particularly where it is wet and warm. *Salmonella* is frequently isolated from many different water sources, including farm effluents, human sewage, and polluted water after several months (6). *Salmonella* Cholera-suis has been isolated after several months from feces or fecal slurries (4,6). Livestock can become carriers of some serovars of *Salmonella* for years, causing no symptoms in the animal (6).

Feces from animals carrying *Salmonella* may contaminate the animals’ water or food supply, thereby infecting other animals. Birds and rodents have been shown to spread *Salmonella* to uninfected livestock, and cats can sometimes acquire *Salmonella* after feeding on infected birds or spending time near bird feeders. Many chicks and young birds carry *Salmonella* as part of their normal intestinal flora. Animals in petting zoos may also serve as sources of infection, even if the animal appears to be healthy. People should always wash their hands immediately after contact with pets or pet feces, or after handling a reptile or bird, and adults should assure that children wash their hands after handling a pet or after touching its environment. See Table 2, Risk Factors for *Salmonella* Infection.

In animals, asymptomatic *Salmonella* infections are common. Overall, approximately 1-3% of domestic animals are thought to carry *Salmonella* spp. but the prevalence can be much higher in some species. Estimates of the *Salmonella* carrier rate among reptiles vary from 36% to more than 80-90%, and several serovars can be found in a single animal (6). High prevalence rates can also be present in some birds and mammals. *Salmonella* spp. has been isolated from 41% of turkeys tested in California (7). *Salmonella* spp. has also been isolated from 1-36% of healthy dogs and 1-18% of healthy cats in various studies, as well as 6% of beef cattle in feedlots (6).

Infected food handlers have been shown to transmit *Salmonella* and have been responsible for outbreaks. Workers who have been ill can shed *Salmonella* for a median of 30 days (range, 2 days to 280 days); therefore, assessment of food-worker infection is essential for
controlling outbreaks traced to restaurants (6). Those carriers in sensitive occupations, such as food handlers, health care workers, teachers, and babysitters, must practice especially good hygiene to avoid infecting others. In some cases, workers are barred from returning to work by the local health authorities until their stool cultures no longer yield *Salmonella*. Some outbreaks caused by cheese, icings, salads, and cold sandwiches have been traced to infected food-workers (6).

**E. ILLNESS/SYMPTOMS OF SALMONELLA GASTROENTERITIS**

After ingestion of contaminated food or water, there is usually a short incubation period of 8 hours or as long as 72 hours (an average of 18 hours), while the organism begins to multiply in the intestinal lumen, causing gastroenteritis. The symptoms of foodborne salmonellosis can vary from a mild, self-limiting gastroenteritis to septicemia (bacteria in the bloodstream leading to shock, fever, and organ failure) that can require hospitalization (4).

Generally, symptoms of *Salmonella* gastroenteritis include an acute, sudden onset of nausea and vomiting, chills, abdominal cramping, diarrhea that is often muco-purulent (containing mucus and pus) and bloody, headache, and myalgia (muscle pain). A headache, rash over the trunk of the body, and a mild fever may also be seen, but these symptoms generally abate in 72 hours. Usually the diarrhea is self-limited, lasting 4-7 days, and the majority of persons recover without treatment. In some cases, the patient may describe the illness only as a minor “stomach flu.” However, in some persons, the diarrhea may be so severe that the patient needs to be hospitalized due to severe dehydration, particularly infants, the elderly, or the immunocompromised. For the most part, the symptoms of foodborne *Salmonella* infection resemble the symptoms due to other foodborne pathogens, so a bacterial culture of stool is necessary to identify the cause. Some people report abnormal bowel movements for up to one year after infection (6). See Table 4, Clinical Features of Salmonellosis.

Whether *Salmonella* remains in the intestine or disseminates into the bloodstream and initiates secondary bacterial infections depends on a variety of host factors as well as the virulence of the strain. All serovars of *Salmonella* can produce all forms of salmonellosis, although a given serovar is often associated with a specific syndrome. For example, salmonellosis acquired from reptiles (often *S*. Paratyphi B, or *S*. Pomona) is often very severe, and may be fatal due to secondary septicemia or meningitis. See Section F, Complications of *Salmonella* Gastroenteritis. Most cases of reptile-associated salmonellosis are seen in children under 10 or people who are immunocompromised (3,6).

Chronic carriers after infection from foodborne salmonellosis are rare, although many convalescent patients excrete small numbers of *Salmonella* in their feces for as long as 4 to 5 weeks, and 45% of children younger than 5 may excrete *Salmonella* 12 weeks after infection, compared to 5% of older children and adults. See Table 4. Clinical Features of Salmonellosis. Some individuals who have had *Salmonella* gastroenteritis may develop late sequelae such as secondary infections or Reiter’s syndrome. See Section F, Complications of *Salmonella* Gastroenteritis.

**F. COMPLICATIONS OF SALMONELLA GASTROENTERITIS**

Although *Salmonella* gastroenteritis is generally self-limiting and usually not life-threatening, in certain people — especially infants and young children, older adults, transplant recipients, pregnant women, and people with weakened immune systems — the development of secondary *Salmonella* infections can be dangerous.
In rare instances, *Salmonella* may spread from the intestinal tract and establish secondary infections in other body sites and cause death. Once *Salmonella* escapes through the intestinal wall and invades the bloodstream, secondary focal infections may occur throughout the body, such as in the tissues surrounding the brain and spinal cord (meningitis), the lining of the heart or valves (endocarditis), soft tissue abscesses, bone abscesses (osteomyelitis), cholecystitis (inflammation of the gallbladder), chronic dyspepsia or irritable bowel syndrome, or in the lungs, causing pneumonia (4). In 1980, for example, we reported a case of disseminated *Salmonella heidelberg* infection causing pneumonia in a patient with carcinoma of the lung (Berkeley, D. and Mangels, J., Amer. Jour. Clin. Path. 74:476-478, 1980). Pneumonia due to *Salmonella heidelberg* had not been previously documented in the literature. Two weeks prior to the development of the patient’s pneumonia, the patient had *Salmonella* gastroenteritis due to the same serovar of *S. heidelberg*.

According to the Centers for Disease Control and Prevention (CDC), pregnant women and infants are particularly vulnerable to complications due to *Salmonella* gastroenteritis (3). Pregnant women experience more severe symptoms and appear to be at higher risk of metastatic secondary infections. Transplacental *Salmonella* infections have been associated with miscarriage and neonatal sepsis (4).

Approximately 400 fatal cases of salmonellosis are reported each year in the U.S. (1,2,6). The overall mortality rate for most forms of salmonellosis is less than 1%; however, some serovars or syndromes are more likely to be fatal. In hospitals or nursing home outbreaks, the mortality rate due to *Salmonella* is approximately 3.6% (1,2).

Another complication of *Salmonella* gastroenteritis is reactive or septic arthritis, called Reiter’s syndrome. If a patient develops Reiter’s syndrome, it usually occurs within 2 to 3 weeks after the initial gastroenteritis, but can range from 4 to 35 days after the initial onset of gastrointestinal symptoms (4). The initial presentation in patients with Reiter’s syndrome is urinary symptoms, such as burning pain on urination (dysuria) or an increased frequency of urination. Other urogenital problems may occur, such as prostatitis in men and cervicitis in women. Then mild to severe joint pain generally occurs, most commonly affecting large weight-bearing joints such as the knees and the lower back (due to involvement of the sacroiliac joint), but other joints can be inflamed as well. Bacterial cultures of the joint (synovial) fluid of patients with Reiter’s syndrome are negative. Conjunctivitis (inflammation of the eyes), including redness of the eyes, eye pain and irritation, or blurred vision, occurs in about 50% of men. Eye involvement typically occurs early in the course of reactive arthritis, and symptoms may come and go. The patient may also suffer from small, superficial ulcers, often causing recurrent stomatitis (inflammation of the mucus lining) in the oral cavity, including the cheeks, gums, tongue, lips, and throat.

Reiter’s syndrome is an autoimmune disorder that develops in response to the inflammation of *Salmonella* gastroenteritis. It occurs in persons with a genetic predisposition to make a cell surface marker known as HLA-B27, which resembles *Salmonella* bacteria as well as a few other organisms, such as *Chlamydia*, *Neisseria gonorrhea*, *Shigella*, or *Campylobacter*. After the initial *Salmonella* infection, the immune system may be triggered to make antibodies to the bacteria and also to the patient’s own cells that have the HLA-B27 marker. The exact mechanism of interaction between the infecting organism and the host is unknown, but it is believed that the over-stimulated autoimmune response becomes deposited in the joints and other areas of the body.
Reiter’s syndrome most commonly strikes individuals aged 20–40 years of age, is more common in men than in women, and is more common in whites than in blacks, owing to the high frequency of the HLA-B27 gene in the white population. Patients with HIV have an increased risk of developing Reiter’s syndrome as well. Reiter’s syndrome occurs in approximately 2% of cases of salmonellosis (4). By the time the patient presents with symptoms of Reiter’s syndrome, the "trigger" infection has been cured or gone away, often making determination of the initial cause difficult. The resulting joint pain and inflammation can resolve completely over 3 to 4 months, or in some cases permanent joint damage can occur. Approximately half of all patients experience transient relapses for several years, and in some rare patients, life-long chronic arthritis can occur. Antibiotic treatment does not make a difference in whether the person later develops arthritis.

G. MICROBIOLOGY OF SALMONELLA

Salmonella are Gram-negative, non-spore-forming bacilli that resemble by size and shape all other Enterobacteriaceae. They are motile, except for Salmonella Gallinarum-pullorum, which is a poultry pathogen. Salmonella have a width of 0.7 to 1.5 µm, a length from 2 to 5 µm, and flagella in all directions surrounding the cell wall (i.e. peritrichous). They are chemoorganotrophs (obtaining their energy from oxidation and reduction reactions of organic matter), and are facultative anaerobes (capable of growing under both aerobic and anaerobic conditions) (4).

Biochemical Characteristics. Most species of Salmonella produce hydrogen sulfide, which can be detected by growing Salmonella on media containing ferrous sulfate, such as TSI, or on solid media, such as HE and XLD. See Table 5. Commonly Used Media for the Isolation of Salmonella. Salmonella are indole production negative, Voges-Proskauer test negative, produce no growth in KCN broth, are urease negative, and are ornithine decarboxylase and lysine decarboxylase positive (4,8). Salmonella reduce nitrates and do not produce cytochrome oxidase. Fermentation of lactose is generally considered to be negative; however, there a few reports of some serovars being lactose positive or weakly positive (4,8). The presence of lactose-fermenting Salmonella strains from clinical samples can present problems in detection and identification.

Serotyping. In addition to biochemical characterization of Salmonella, complete identification of Salmonella requires serological identification (serotyping) for confirmation. Antisera are available commercially from a variety of sources, e.g., BD Diagnostics, Difco Laboratories, Hardy Diagnostics, Remel, Oxoid, and others.

Salmonella serotyping is a method based on the immunologic characterization of the three surface structures: O antigen, a carbohydrate antigen which is the outermost portion of the lipopolysaccharide layer covering the bacterial cell; H antigen, a protein antigen of the bacterial flagella called flagellin; and Vi antigen, a heat-labile capsular polysaccharide present in specific serotypes, primarily in S. Typhi.

H. PATHOGENIC MECHANISMS OF SALMONELLA

Salmonella first enters the stomach with contaminated food or water, where it has the ability to survive the host’s normal stomach acidity. Salmonella then passes on to colonize the ileum and colon, where the organism adheres to intestinal epithelial cells by adhesive structures called fimbriae. Salmonella is capable of invading the intestinal epithelial cells of the jejunum, the ileum, and colon to initiate gastroenteritis with as few as 200 organisms (4). In these sites,
tissue macrophages engulf *Salmonella* by phagocytosis; however, instead of being typically destroyed by the macrophage, *Salmonella* is capable of multiplying inside the cell and then begins to produce a cholera-like endotoxin. The endotoxin causes chills and fever, and disrupts and destroys the lining of the bowel, causing increased fluid production and profuse diarrhea.

The initial pathogenic mechanism of *Salmonella* is due to the virulence of the lipopolysaccharide outer cell membrane. Lipopolysaccharides (LPS) are large molecules consisting of a lipid and a polysaccharide found in Gram-negative bacteria. LPS elicits a strong immune response, causing human cell damage and leading to the production of diarrhea, and permits further invasion of *Salmonella* into the intestinal epithelial cells.

Recent research has shown that *Salmonella* has additional virulence factors called *Salmonella* pathogenicity islands (SPIs) (4). The SPIs are unique pathogenic factors containing genes specifically associated with *Salmonella*. The protein products of these genes are injected directly into the human host cell by syringe-like organelles on the surface of *Salmonella*. These specific proteins ultimately manipulate the cellular functions of the infected host cell, permitting bacterial intracellular replication, bacterial survival and host cell damage, and facilitating the progression of the infection. The SPIs of *Salmonella* also express several enzymes that inactivate reactive oxygen and nitrogen produced by the host’s macrophages that normally aid in the destruction of the organism. The enzymes produced from the SPIs permit bacterial survival within the host cell and contribute to the virulence of the organism.

I. DIAGNOSIS AND IDENTIFICATION OF SALMONELLA GASTROENTERITIS

Since many agents of foodborne infection cause similar symptoms of gastroenteritis in humans, the diagnosis of *Salmonella* gastroenteritis is dependent upon isolating the organism from stool. A fresh fecal specimen (less than 2 hr) should be submitted to the clinical laboratory from patients with symptoms of gastroenteritis. The stool sample should be collected during active diarrhea, preferably as soon as possible after the onset of illness. Stool samples (1 mL of liquid or 1 marble-sized piece of whole fresh stool) should be submitted in leak-proof, wide-mouth containers. If a delay of more than 2 hours is anticipated, the stool sample should be placed in semi-solid modified Cary-Blair transport medium (4,8). Modified Cary-Blair medium contains reducing agents and appears to be the most suitable single transport medium for *Salmonella* as well as for other bacterial enteric pathogens (4,8). Modified Cary-Blair medium is available from BD Biosciences, Hardy Diagnostics, Oxoid, Remel, and others. Specimens received in the transport medium should be processed immediately, or stored at 4°C until processed; it is best to process the stool sample from the transport medium within 48 hours. In an acute infection, there are usually a very high number of organisms in the stool, so testing of a single stool sample has a high sensitivity for detection of *Salmonella*. Stool specimens on patients with *Salmonella* gastroenteritis may continue to yield low numbers of organisms for several weeks.

**Rejection Criteria.** Stool specimens not in transport medium and received more than 2 hours after collection should be rejected. If the specimen in transport medium is delayed for more than 3 days at 4°C, or is delayed for more than 24 h at 25°C, reject the specimen and request recollection since yield will be compromised (4). On hospitalized patients, the 3-day rule (rejection of specimens for patients who have been in the hospital for more than 3 days) should be used as a criterion for acceptability of routine inpatient stool culture requests (4,8). Do not perform a culture on formed, hard or solid stools, since patients who have gastroenteritis due to *Salmonella* or other enteric pathogens will not have a formed stool.
**Media.** Freshly passed stool, or stool samples from modified Cary-Blair transport medium, should be inoculated directly onto a variety of differential and selective agar plates. Many plating media, varying from low selective to highly selective, are available for isolating *Salmonella* from fecal specimens. See Table 5, Commonly Used Media for the Isolation of *Salmonella*. Media of low selectivity include MacConkey (MAC) agar and eosin methylene blue (EMB) agar. Media of intermediate selectivity include xylose-lysine-deoxycholate (XLD), deoxycholate citrate agar (DCA), Salmonella-Shigella (SS) agar, or Hektoen Enteric (HE): all are widely used to screen for *Salmonella* from clinical specimens. Highly selective media include bismuth sulfite agar and brilliant green agar are used for detecting carriers or used during outbreaks. Bismuth sulfite or brilliant green agar are seldom used under routine circumstances because they are inhibitory for other bacterial pathogens likely to cause gastroenteritis (4). Most laboratories today use HE or XLD in combination with either MAC or EMB because these media may also be used for the isolation of *Shigella* species. It is recommended that a combination of media with low selectivity and intermediate selectivity be used to recover stool pathogens (4,8). Colonies of *Salmonella* are usually detected after 24 hours of incubation at 35°C.

**Enrichment broth.** The use of enrichment broth may increase the probability of isolating *Salmonella* from fecal specimens by suppressing competing organisms, although in most circumstances the isolation of *Salmonella* from acutely ill persons is generally possible by direct plating of specimens (4,8). Tetrathionate broth, selenite broth, or GN broth can be used to aid recovery of low numbers of organisms. See Table 5, Commonly Used Media for the Isolation of *Salmonella*. Be aware, however, that enrichment broths for *Salmonella* are usually highly selective and can inhibit certain serotypes of *Salmonella*, particularly *Salmonella* Typhi. Stool specimens are inoculated directly into enrichment broths and then subcultured onto media after incubation. Keep in mind that GN broth requires subculture to media at 6 to 8 hours—not after overnight incubation, whereas selenite or tetrathionate broth should be subcultured at 18 to 24 hours of incubation. If enrichment broths are incubated for too long, the nonpathogenic enteric bacteria can overgrow the pathogens and negate the value of the procedure. The usual procedure is to subculture the broth to one or two different selective and differential media. Selenite broth is used for *Salmonella* enrichment, while GN broth can be used for both *Salmonella* and *Shigella* enrichment. Note: laboratories that have historical data showing very poor recovery of additional pathogens not seen on initial primary plates can make a case for abandoning the routine use of enrichment broths. Generally, enrichment broth increases the yield of Salmonella by 10% (4,8).

**Chromogenic Media for Salmonella.** The use of chromogenic media permits the user to rapidly distinguish pathogens from non-pathogenic enteric bacteria based on colony color. Chromogenic media can be used along with other previously described media for primary isolation of stool pathogens from clinical specimens and lessens the need for secondary confirmatory testing and the time to identification. Chromogenic media are available from BD Biosciences, Hardy Diagnostics, Oxoid, Remel, and others. See Table 5, Commonly Used Media for the Isolation of *Salmonella*. There are chromogenic media for the screening of only *Salmonella*, or there are other chromogenic media for the screening of both *Salmonella* and *Shigella*. Differentiation of *Salmonella* from non-pathogenic bacteria is accomplished by three mechanisms: chromogenic reactions, carbohydrate fermentation, and hydrogen sulfide production. Depending upon the choice of chromogenic medium used and the manufacturer, *Salmonella* may produce deep pink to magenta colored colonies with black centers indicating the production of H₂S.
Approach to identification/workup of potential pathogens. Screen primary selective and differential plates for lactose-negative and /or H₂S positive colonies at 24 hours. Work up potential pathogens by picking one representative colony of each suspicious morphologic type. For example, from XLSD media pick one representative colony of each morphologic type of red to red-orange colony and any colony with a black center. From HE media, pick one representative colony of each morphologic type of green and blue colonies that are not pinpoint and any colony with a black center. From MAC or EMB media, pick one colorless or transparent colony.

Biochemical Testing. Subculture each suspect colony to BAP, KIA or TSI, and urea agar for screening identification. The use of commercial kit identification systems initially on suspect colonies, rather than screening with individual biochemical tests, may be done depending upon the work-flow or the budget of the laboratory. On KIA or TSI, most Salmonella strains produce an Alk/AG+ H₂S + (an alkaline slant with acid and gas in the butt (bottom) of the tube with black in the medium) indicating that lactose is not fermented, and the organism produces gas and H₂S in the butt of the tube. Salmonella are urease negative, so urea agar would produce no color change in the media. Lysine iron agar (LIA) is also a useful screening medium because most Salmonella isolates, even those rare strains that ferment lactose, decarboxylate lysine. This would be evident on LIA agar as a purple slant and purple butt and produce H₂S (black in the butt of the tube) (8).

After 18 to 24 hours of incubation, read the biochemical tests. Test for agglutination for Salmonella polyvalent (O) antigens when the screening or kit biochemical tests are consistent with a possible Salmonella species. Generally, clinical laboratories identify the organism to genus biochemically and may carry out limited serologic typing assays to place the organism into a group. Isolates are forwarded to a public health laboratory. If the biochemical reactions for a particular isolate are not characteristic, but Salmonella antigens are found, the isolate should be plated on MAC or EMB to obtain a pure culture, tested with a complete set of biochemicals tests, or forwarded to a public health or reference laboratory. If the use of biochemicals identifies the isolate as Salmonella, but it fails to serogroup, repeat the typing using a boiled suspension of the organism in saline. If typing is not available, submit all Salmonella to the health department for confirmatory identification and for typing. Do not perform susceptibility testing on Salmonella, except when the organism is isolated from blood-stream infection, or from normally sterile body sites, since treatment may prolong the carrier state or lead to a higher rate of clinical relapse (4,8).

Serologic Testing. Salmonella serologic testing procedures are used in conjunction with biochemical tests to confirm the identity of the organism and for differentiating strains of common Salmonella serotypes.

The approach most commonly used for determining O antigens is to initially test the isolate by slide agglutination in antisera against polyvalent O groups A to E₁ because approximately 95% of Salmonella isolates belong to one of these O groups. Antisera are available from BD Bioscience, Difco, Hardy Diagnostics, Remel, and others. Polyvalent O serogroup determination is adequate for confirmation of isolates as Salmonella. Generally, clinical laboratories identify the organism to genus biochemically and may carry out limited serologic typing assays and then submit isolates to a public health laboratory. Full serotype determination is useful for public health surveillance because the serotype of an isolate often correlates with a particular disease syndrome of food vehicles, making serotype data particularly useful in identifying cases and defining outbreaks.
Serotyping can be accomplished by a slide agglutination test. The slide agglutination test is performed by mixing 1 drop of a milky suspension of the organism prepared in physiological saline with 1 drop of polyvalent O (somatic surface antigen) liquid antiserum. Rotate slide for 1 min and observe for agglutination (clumping). A positive test for *Salmonella* is when the polyvalent O somatic antiserum agglutinates (clumps) in the presence of homologous antiserum, indicating that the strain contains O antigen.

All isolates that biochemically resemble *Salmonella* but fail to agglutinate in the polyvalent antisera should be boiled for 15 to 30 minutes and re-tested after cooling. This is accomplished by making a heavy suspension of the organism in saline and placing the tube of saline in a water bath. Some *Salmonella* with either capsules or flagella can give a false-negative result (falsely produce no agglutination). Also, the presence of the Vi antigen can present a problem in accurate serotype determination. The Vi capsular antigen can block the binding of antibodies against O antigens. If slide agglutination studies are positive for the Vi antigen, but negative for the O antigen, the suspension should be heated in boiling water for 15 to 30 minutes and retested. This procedure removes the Vi capsular polysaccharide to permit accurate serotyping. Isolates that biochemically resemble *Salmonella* but fail to agglutinate in any typing sera should be sent to a reference or public health laboratory for definitive identification. Serological identification should always be confirmed with biochemical testing because serotypes can be shared or cross-react with other organisms.

Clinical laboratories may issue a preliminary report of *Salmonella* when an isolate is positive either with *Salmonella* O group antisera or by biochemical identification methods, preferably when positive by both methods. On hospitalized patients, notify infection control personnel and the nursing care unit immediately by telephone with presumptive identification. For patients who have been discharged from the hospital or whose specimens were received as outpatient specimens, communicate the presumptive identification report to the physician responsible for the patient’s care. Laboratories should follow the procedures recommended by their state health departments for submitting isolates for further characterization, and complete serotyping.

**Specialized Tests.** A latex agglutination kit is available for screening *Salmonella* from selenite enrichment broth or for screening individual colonies directly from primary plates (Wellcolex Colour Salmonella; available from Hardy Diagnostics, Oxoid, and Remel). Wellcolex testing directly from GN broth has been found to be less sensitive than from selenite broth so is not recommended (4,8). When using Wellcolex Colour Salmonella, keep in mind that it identifies only those *Salmonella* isolates belonging to the more common O serogroups. Wellcolex Colour Salmonella is a useful rapid screening test that can be used to eliminate *Salmonella*-negative samples from further testing. The test allows negative results to be reported at least 24 hours earlier than by conventional methods.

There are other specialized tests used in the food industry or to test environmental samples for *Salmonella*, but they have not been approved by the FDA for clinical laboratory use. Some of these rapid tests for *Salmonella* include EIA, DNA probes, or real time or PCR techniques. Some DNA amplification techniques, such as PCR, are used in public health laboratories to rapidly identify *Salmonella*.

**J. TREATMENT OF SALMONELLA GASTROENTERITIS**

Since *Salmonella* gastroenteritis is usually self-limiting, almost all persons infected with *Salmonella* recover without any specific antibiotic treatment. Antimicrobial therapy is not recommended for uncomplicated *Salmonella* gastroenteritis, and routine susceptibility testing of
fecal isolates is not warranted for treatment purposes. Unless patients are acutely ill with enteric fever, have a bloodstream infection, or if *Salmonella* is isolated from a normally sterile body site, antibiotic treatment is not indicated. Antibiotic treatment does not decrease the length of illness, but may prolong the carrier state, increase the risk of relapse, and encourage the emergence of resistant strains (4). Many species of *Salmonella* have become resistant to antibiotics largely as a result of the wide use of antibiotics to promote the growth of animals used for food (3).

Supportive measures, such as fluid and electrolyte replacement to prevent dehydration and electrolyte imbalance, are the principal therapies for most patients with *Salmonella*. Patients should drink extra fluid as long as the diarrhea lasts. Severely dehydrated patients should receive rapid blood volume expansion with intravenous fluids. Antimotility agents, such as loperamide, can lead to prolonged illness or intestinal perforation in any invasive diarrhea, and should be avoided (4).

**K. HOW TO PREVENT SALMONELLA GASTROENTERITIS**

Since *Salmonella* is spread through fecal-contaminated food, or spread by direct contact with contaminated animal feces, prevention of *Salmonella* gastroenteritis is achieved by reducing exposure to potentially contaminated food sources and reducing exposure to contaminated animal feces. See Table 6, Tips to Prevent *Salmonella* Infection.

Any food of animal origin or any vegetable may be contaminated with *Salmonella*. People should not eat raw or undercooked eggs, poultry, or meat. Chickens and turkeys contaminated with *Salmonella* from their intestinal tracts during processing can serve as a vehicle of food-borne infection if they are not fully cooked. Meat, including hamburgers, should be well-cooked, not pink in the middle. If you are served undercooked meat, poultry, or eggs in a restaurant, send it back for further cooking. People should not consume raw or unpasteurized milk or other unpasteurized dairy products, such as unpasteurized cheese, sour cream, whipping cream, and the like. Raw eggs may be unrecognized in some foods such as homemade hollandaise sauce, Caesar and other homemade salad dressings, tiramisu, homemade ice cream, homemade mayonnaise, cookie dough, and frostings; these items should be avoided (3,5,6). Vegetables and fruit should be thoroughly washed before consumption. See Table 6, Tips to Prevent *Salmonella* Infection.

Eggs should be handled properly. Shell eggs are safest when they are stored in the refrigerator until needed, then thoroughly cooked and promptly consumed. Keeping eggs adequately refrigerated prevents any *Salmonella* present in the eggs from growing to greater numbers. Cooking reduces the number of bacteria present in an egg; however, an egg with a runny yolk still poses a greater risk than does a completely cooked egg. *Salmonella* are killed in poultry, in other meat, and in other food products after being heated to 74 °C (165 °F) for one hour.

Care must be taken during food preparation to prevent cross-contamination from meat, poultry, and uncooked eggs to other food items. See Table 6, Tips to Prevent *Salmonella* Infection. Reduce the risk of cross contamination by thoroughly cleaning knives, cutting boards, other utensils, and counters. All surfaces and utensils that contact uncooked meat or poultry should be washed after, and especially before, using with other foods that are to be eaten without cooking. Uncooked meats should be kept separate from produce and cooked foods, to prevent cross-contamination. Hands should be washed before handling food, and between handling different food items, particularly after touching uncooked meat or poultry. Proper food thawing
and handling techniques, along with proper sanitation, are important to prevent Salmonella gastroenteritis.

Handling and touching pets and animals in petting zoos can be a source of Salmonella infection. It is essential to wash hands after contact with animals, baby poultry, reptiles, dogs, cats, and animals in petting zoos to reduce the risk of acquiring Salmonella. This is particularly important for children. People should wash their hands after contact with animal feces. See Table 6, Tips to Prevent Salmonella Infections. Since reptiles are often carriers of Salmonella, and the organism can contaminate both their skin and their environment, it is essential that people should immediately wash their hands after handling reptiles. Reptiles (including turtles) are not appropriate pets for small children and should not be in the same house as an infant. Salmonella carried in the intestines of chicks and ducklings can contaminate their environment and the entire surface of the animal, so children can be exposed to the bacteria by simply holding or cuddling the birds. People should immediately wash their hands after touching birds, including baby chicks and ducklings, or items from their environment.

L. CONCLUSIONS

The symptoms of Salmonella gastroenteritis have been recognized since at least the time of Alexander the Great in 323 B.C. Subsequent incidents of Salmonella foodborne gastroenteritis have been reported during wars, disasters, crowding, and famines. However, the large foodborne outbreaks that we experience today result from the way food is produced and distributed on an industrial scale. Food can be distributed throughout the U.S. and the world in a short period of time. Salmonella is the most frequently diagnosed bacterial cause of human gastroenteritis in the U.S. It is estimated that Salmonella infects over 1.4 million people each year and that many cases go undiagnosed.

The name Salmonella was given to the genus in 1885 to recognize the work of Dr. Daniel Salmon, who first isolated and identified the source of hog cholera as Salmonella Cholera-suis. The nomenclature today is confusing because of the many serovars (antigenic variations) of Salmonella that can be classified according to their somatic (O) and flagellar (H) antigens. Further, the nomenclature of Salmonella is evolving because of the use of various molecular techniques that have changed the species classification of many of the strains of Salmonella. However, most clinical laboratories and infectious disease specialists continue to use the simple rather than serological or molecular nomenclature to describe Salmonella species.

Most people infected with Salmonella develop diarrhea, a mild fever, and abdominal cramps 8 to 72 hours after ingestion of contaminated food. Often the illness can be accompanied by nausea and vomiting. Generally the symptoms last 5 to 7 days and resolve on their own without medical treatment. Usually the gastroenteritis is self-limiting, although in some cases the organism can spread to the bloodstream and cause secondary infections in the endocardium, lungs, bones, joints, kidneys, and soft tissues. One severe complication of Salmonella gastroenteritis that may occur within 2-3 weeks after the initial gastroenteritis is Reiter’s syndrome. Patients develop urinary symptoms, severe joint pain and inflammation of the eyes. Reiter’s syndrome is an autoimmune disorder that develops in individuals who have the HLA-B27 marker.

Salmonella are transmitted to humans by eating food or drinking water contaminated with animal feces. The organism can be part of the normal flora of poultry, wild birds, cattle, swine, reptiles where it may not cause disease in these animals, but can cause gastroenteritis in man. Other animals that serve as source of the infection are chicks, ducklings, dogs, cats, kittens, and
puppies. Usually the contaminated food is of animal origin such as poultry, beef, eggs, unpasteurized milk and dairy products. Recently there has been an increase in *Salmonella* gastroenteritis associated with consumption of fresh fruits and vegetables.

In 2013, there were 7,800 cases of *Salmonella*, representing about 40% of the foodborne gastroenteritis reported to FoodNet. There were 4,563 hospitalizations and 68 deaths, resulting in an estimated $365 million in direct medical cost. There is a marked seasonality in the rate of *Salmonella* infection in the United States; the highest rate of infection occurs during March through October.

The diagnosis of *Salmonella* is dependent upon isolating the organism from stool samples, using various selective and differential media to aid in the recovery of the organism, including media such as MAC, EMB, HE, or XLD. If biochemical tests are consistent with *Salmonella*, serologic testing with O polyvalent antisera is used. *Salmonella* are Gram-negative rods that are urease negative, H2S positive, lactose negative, indole negative, VP broth negative, show no growth in KCN broth, and are lysine positive.

Antibiotic treatment is not necessary for salmonellosis since most cases are self-limiting unless the organism has progressed beyond the intestinal tract. In fact, antimicrobial treatment does not decrease the length of illness and was associated with an increased risk of positive stool culture after 3 weeks (prolonged the carrier state), increased risk of relapse, and adverse drug reactions.

Prevention of *Salmonella* gastroenteritis can be accomplished by avoiding raw or undercooked poultry, eggs, and beef, by washing vegetables, by washing hands after contact with animal feces, and by avoiding unpasteurized milk or dairy products. Cross-contamination in the kitchen can be avoided by washing hands, cutting boards, counters, knives and other utensils after they have touched uncooked meat or poultry.

M. REFERENCES


http://www.CDPH.ca.gov/HealthInfo/discond/Pages/Salmonellosis.aspx


http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6315a3.htm?s_cid+mm6315a3_w
Table 3. Animal Sources of *Salmonella* Infection*

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<td>Cattle</td>
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<td>Poultry (including chicks and ducklings)</td>
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<td>Sheep</td>
<td>Tortoises</td>
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<td>Pigs</td>
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<td>Birds</td>
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<td>Dogs and cats (including puppies and kittens)</td>
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Table 4. Clinical Features of Salmonellosis*

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<tr>
<th>Incubation Period</th>
<th>8-72 hours after ingestion of contaminated food or water.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presentation</td>
<td>Diarrhea, fever, abdominal cramps, vomiting.</td>
</tr>
<tr>
<td></td>
<td>Stools are loose and can be watery, with small volume of blood.</td>
</tr>
<tr>
<td></td>
<td>Often mild fever that lasts only a few days.</td>
</tr>
<tr>
<td></td>
<td>Headache, myalgias (muscle pain), and other systemic symptoms can also occur.</td>
</tr>
<tr>
<td>Duration of Illness</td>
<td>4-7 days</td>
</tr>
<tr>
<td></td>
<td>The organism may be carried in the GI tract, with a mean duration of 4-5 weeks. 45% of children younger than age 5 excrete <em>Salmonella</em> up to 12 weeks after infection, compared with 5% of older children and adults. It may take several months for the patient’s bowels to return to normal.</td>
</tr>
<tr>
<td>Complications</td>
<td>Cholecystitis, bacteremia, meningitis, secondary focal infections and endocarditis.</td>
</tr>
<tr>
<td></td>
<td>Reiter’s syndrome can occur after 2-3 weeks.</td>
</tr>
<tr>
<td></td>
<td>Chronic dyspepsia or irritable bowel syndrome.</td>
</tr>
</tbody>
</table>

Table 5. Commonly Used Media for the Isolation of *Salmonella*

<table>
<thead>
<tr>
<th>Medium</th>
<th>Inhibitors</th>
<th>Feature/Morphology of <em>Salmonella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hektoen Enteric (HE)</td>
<td>Bile salts, ferric ammonium citrate, bromthymol blue, fuchsin.</td>
<td><em>Salmonella</em> is blue or green, and may have black centers (H₂S).</td>
</tr>
<tr>
<td>Deoxycholate Citrate Agar (DCA)</td>
<td>Sodium deoxycholate, sodium citrate, ferric citrate, neutral red.</td>
<td><em>Salmonella</em> is colorless, and may have black centers (H₂S).</td>
</tr>
<tr>
<td>MacConkey (MAC)</td>
<td>Bile salts, crystal violet, neutral red.</td>
<td><em>Salmonella</em> is colorless or transparent.</td>
</tr>
<tr>
<td>Salmonella-Shigella agar (SS)</td>
<td>Bile salts, citrate, thiosulfate, ferric citrate, brilliant green, neutral red.</td>
<td><em>Salmonella</em> is colorless or transparent; may have black centers (H₂S).</td>
</tr>
<tr>
<td>Xylose Lysine Deoxycholate agar (XLD)</td>
<td>Deoxycholate, thiosulfate, ferric ammonium citrate, xylose, lactose, sucrose, phenol red.</td>
<td><em>Salmonella</em> is red, some with black centers (H₂S).</td>
</tr>
<tr>
<td>Chromogenic Salmonella agar</td>
<td>Bile salts, deoxycholate, sodium thiosulfate, proprietry inhibitors.</td>
<td>Color of <em>Salmonella</em> depends on chromogens used in media. Often is pink to magenta with black centers (H₂S).</td>
</tr>
<tr>
<td>Gram-negative (GN) broth</td>
<td>Deoxycholate, citrate, dextrose, mannitol.</td>
<td>Enhances growth of mannitol-fermenting rods such as <em>Salmonella</em> and <em>Shigella</em>. Subculture at 6-8 hrs.</td>
</tr>
<tr>
<td>Selenite F</td>
<td>Sodium selenite, sodium phosphate.</td>
<td>Subculture at 18-24 hrs.</td>
</tr>
</tbody>
</table>

Table 6. Tips to Prevent *Salmonella* Infection*

1. Cook poultry, ground beef, and eggs thoroughly. Be sure the meat is cooked throughout; i.e., no longer pink and any juices should run clear. All poultry should be cooked to reach a minimum internal temperature of 165°F.
2. If you are served undercooked meat, poultry, or eggs in a restaurant, send it back for further cooking.
3. Wash raw vegetables and fruit thoroughly before eating.
4. Wash hands with soap after handling reptiles, snakes, birds, baby chicks, or ducklings, and after contact with pet feces.
5. Wash hands with soap and water after contact with animals in a petting zoo.
6. Avoid unpasteurized milk, unpasteurized milk products, and unpasteurized juices.
7. Avoid sprouts.
8. Avoid eating raw eggs in homemade ice cream, eggnog, homemade mayonnaise, homemade salad dressings such as Hollandaise sauce, tiramisu, cookie dough, and homemade frostings.
9. Wash hands and cooking utensils with soap and warm water after contact with meat, poultry, or raw eggs.
10. Wash cutting boards, counters, knives, and other utensils thoroughly after they have come in contact with uncooked foods.
11. Do not cross-contaminate foods. Keep uncooked meat and poultry separate from produce and other refrigerated items. Store and wrap food items separately.

REVIEW QUESTIONS
Course #DL-003
Choose the one best answer

1. Which set of symptoms is most typical of Salmonella gastroenteritis:
   a. diarrhea, fever, shock, chills
   b. diarrhea, low blood pressure, high fever, headache
   c. diarrhea, high fever, shock, nausea
   d. diarrhea, cramps, abdominal pain, vomiting

2. The incubation period of Salmonella infection after ingestion of contaminated food is:
   a. 4-6 hours
   b. depends upon food source
   c. 8-72 hours
   d. one week

3. The organism involved in the outbreak associated with clinical and teaching microbiology laboratories:
   a. mutated from a non-pathogenic strain
   b. was restricted to infecting only those working in laboratories
   c. was caused by a commercially available organism
   d. was caused by Salmonella Enteritidis

4. The number of Salmonella organisms required to initiate gastroenteritis is:
   a. 2,000 organisms
   b. 200 organisms
   c. 10^6 organisms
   d. 35 organisms

5. Which set of symptoms is characteristic of Reiter’s syndrome:
   a. dysuria, eye involvement, joint pain
   b. diarrhea, fever, joint pain
   c. dysuria, diarrhea, pneumonia
   d. cervicitis, eye involvement, pneumonia

6. Which pathogenic factor is associated with Salmonella infection:
   a. exotoxins that act as invasive antigens
   b. N-linked glycosylation that induces shock
   c. LPS that neutralize the normal acidity of the stomach
   d. SPIs that manipulate host cell functions

7. The term zoonotic infection means an organism that:
   a. occurs normally in animals but causes infection in humans
   b. occurs in animals and is transmitted in a zoo
   c. is transmitted to animals from humans and causes infection
   d. is pathogenic in animals and cause infection in other animals
8. The GN broth should be subcultured to solid media at the following time:
   a. after 10-12 hours of incubation
   b. after 6-8 hours of incubation
   c. after 18-24 hours of incubation
   d. when you observe growth

9. Fimbriae are important *Salmonella* virulence factors because they:
   a. produce endotoxins
   b. promote the formation of SPIs
   c. act as adhesive structures
   d. contain lipopolysaccharide

10. Which is an incorrect description of Reiter’s syndrome:
    a. an autoimmune response
    b. HLA-B27 is deposited in joints
    c. HLA-B27 resembles *Salmonella*
    d. the immune system is triggered to make antibodies to *Salmonella*

11. Which of the following would be correct according to the new nomenclature for *Salmonella*?
    a. *Salmonella* banana
    b. *Salmonella* cholera-suis
    c. *Salmonella* enteric typhimurium
    d. *Salmonella* Typhimurium

12. The correct biochemical profile for *Salmonella* is:
    a. H$_2$S positive, indole production negative, urease negative
    b. H$_2$S variable, Voges-Proskauer test negative, urease positive
    c. H$_2$S negative, Voges-Proskauer test positive, urease negative
    d. H$_2$S positive, indole production positive, urease positive

13. Three central features for preventing *Salmonella* infection are:
    a. wash hands after handling pets, use separate cutting board for raw meats, avoid buying off-brand raw meat products
    b. use separate cutting board for raw chicken, cook poultry until 74°C, wash hands after contact with pets
    c. cook hamburgers until juices are pink, use only one cutting board, cook eggs until runny
    d. cook poultry until 50°C, avoid cross-contamination in the kitchen, cook eggs until runny

14. Which of the following is not safe to eat?
    a. poultry cooked to 170°F
    b. steamed spinach
    c. commercial mayonnaise
    d. washed sprouts
15. To adequately screen primary selective and differential media for Salmonella you need to pick:
   a. lactose positive and H$_2$S negative colonies
   b. colonies from MAC that are pink to red
   c. colonies from XLD that are yellow to blue
   d. colonies from EMB that are colorless to transparent

16. What is the best term that describes the principle of serotyping for *Salmonella*:
   a. boiling is required to prevent O antigen blockage of antibodies
   b. isolates that fail to agglutinate in polyvalent should be boiled 3-5 minutes
   c. serotyping is based on the immunologic characterization of O and H antigen
   d. biochemical testing is not required if serotyping is performed

17. Which of the following is a characteristic of LPS of *Salmonella*:
   a. promotes adherence to macrophages
   b. manipulates cellular functions
   c. elicits an immune response, causing cell damage
   d. produces enzymes to inactivate reactive oxygen and nitrogen

18. Which of the following statements about Salmonella is not correct:
   a. found in the normal intestinal flora of reptiles
   b. found in poultry cooked until at least 165 °F
   c. found in waste water effluents
   d. found in the normal intestinal flora of poultry

19. Which statement is not a correct characteristic of *Salmonella* pathogenic islands:
   a. they are pathogenic factors that interfere with protein uptake
   b. they are protein products that are injected into host cells
   c. they manipulate host cell functions
   d. they inactivate reactive oxygen and nitrogen

20. Which of the following specimens is acceptable for *Salmonella*:
   a. a formed solid stool
   b. a stool specimen not in transported media, but received after 2 hours
   c. a sample from an inpatient hospitalized more than 3 days
   d. a bloody stool placed in transport media

21. The typical plate morphology of Salmonella on Hektoen Enteric medium is a:
   a. pinpoint yellow colony with black center
   b. green or blue colony
   c. clear colony with red center
   d. red to red-orange colony with black center

22. The CDC believes the actual annual incidence of *Salmonella* gastroenteritis is underestimated because:
   a. only about 3% of cases are reported to the CDC or are laboratory confirmed
b. inaccurate data is submitted from clinical laboratories
c. data is seasonal, so cases during the holidays are not reported
d. the disease is currently reported as viral gastroenteritis by the CDC

23. A reason why antimicrobial therapy is not recommended for *Salmonella* gastroenteritis is:
   a. antibiotics do not decrease the length of illness
   b. antibiotics increase the risk of positive stool culture after 3 weeks
   c. antibiotics block the serological identification of the organism
   d. antibiotics may lead to antimicrobial resistance

24. Extraintestinal infections following *Salmonella* gastroenteritis have not been associated with infections:
   a. in the lungs, causing pneumonia
   b. in the blood stream, causing bacteremia
   c. in the heart, causing endocarditis
   d. of the immune system

25. The Kauffmann and White scheme for *Salmonella* nomenclature is based on:
   a. species names given according to the animal source
   b. DNA hybridization studies to provide serovars
   c. surface antigens to provide serovars based on their O and H antigen types
   d. serovars based on biochemical differences

26. The best way to avoid *Salmonella* gastroenteritis from eggs is to:
   a. consume only brown eggs
   b. wash the exterior of eggs before consuming
   c. refrigerate eggs while you transport them home
   d. avoid consuming raw eggs, even in cookie dough

27. Which statement is incorrect regarding *Salmonella* found in reptiles:
   a. 90% of reptiles may carry *Salmonella* in their intestinal flora
   b. several *Salmonella* serovars can be found in a single animal
   c. pet reptiles do not carry *Salmonella*
   d. some strains from reptiles are more likely to cause severe secondary complications

28. Which pathogenic mechanism is not associated with the virulence of *Salmonella*:
   a. adhesive structures known as fimbriae
   b. adhesive structures known as H antigens
   c. lipopolysaccharide on outer cell wall
   d. *Salmonella* pathogenic islands

29. The definition of the “three day rule” for acceptance of stool samples from inpatients is:
   a. do not accept stool samples from patients who have been in the hospital for more than 3 days
   b. do not accept stool samples from patients who have produced stool samples for more than 3 days
c. do not perform a culture on formed or hard stools for longer than three days

d. do not perform a culture on stool samples unless the patient has been hospitalized for more than three days

30. One tip the CDC recommends to prevent *Salmonella* gastroenteritis is:

a. freeze meat to reduce *Salmonella* contamination

b. clean knives, cutting boards, and counters with an antiseptic

c. vegetables and fruits should be thoroughly washed before consuming

d. all meat should be cooked until pink in the center