# Overview of Food Microbiology

## OBJECTIVES

At the end of this module, you will be able to:

1. Explain the structural similarities and/or differences among Gram-positive and Gram-negative bacteria as well as their isolation and identification using serological, biochemical, and molecular techniques.
2. Identify the functions of the bacterial cell wall.
3. Identify the extrinsic and intrinsic parameters that affect bacterial growth.
4. List the primary sources of microorganisms in meat and poultry products as well as the establishment's environment.
5. Explain the rationale of how food become contaminated and how does it leads to foodborne illnesses.
6. Identify the foodborne pathogens of concern from the public health regulatory and food industry perspectives. Explain their physiology and pathogenicity.
7. Describe how a foodborne outbreak occurs, the methods of detection, and the outcome in food legislation.
8. Define the terms epidemiology, epidemic, and endemic.
9. Identify the surveillance systems for tracking foodborne disease.
10. List the types of food preservation that are currently practiced to control, reduce, or eliminate foodborne pathogens.
11. List the microbiological testing programs conducted by FSIS and the meat and poultry establishments.

## INTRODUCTION

Food microbiology encompasses the study of microorganisms which have both beneficial and deleterious effects on the quality and safety of raw and processed meat, poultry, and egg products. Food microbiology focuses on the general biology of the microorganisms that are found in foods including: their growth characteristics, identification, and pathogenesis. Specifically, areas of interest which concern food microbiology are: food poisoning, food spoilage, food preservation, and food legislation. Pathogens in product, or harmful microorganisms, result in major public health problems in the United States as well as worldwide and is the leading causes of illnesses and death.

It is important for you as a PHV to understand some of these basics because they have an effect on the meat, poultry, and egg products that FSIS regulates. In this module, we'll cover a brief overview of some of the basic principles of food microbiology and explain how they apply to meat, poultry, and egg products. Also we will review the FSIS microbiological sampling programs.

## OVERVIEW OF BASIC MICROBIOLOGY

Let's review, in general, the microbiology basics that you learned in Veterinary School. As a FSIS-PHV public health official it is important for you to understand the dynamics (identification, physiology, pathogenesis, survival, etc) of those pathogens of concern to the food industry and consumers.

As you know microbiology is defined as the science that deals with the study of microorganisms, including algae, bacteria, fungi, protozoa, and viruses. Specifically, bacteria are the most abundant of all organisms, they are unicellular, are relatively small ranging in size from 0.5- to 5.0 μm, and for the most part they reproduce asexually. Although there are bacterial species capable of causing human illness (pathogens) and food spoilage, there are also beneficial species that are essential to good health and the environment (examples: synthesize vitamins, digest plant cellulose, fixing nitrogen in plant roots, etc.).

Every bacterial species have specific nutritional requirements, temperature, humidity, etc for energy generation and cellular biosynthesis. The bacterial cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation and under favorable conditions, a growing bacterial population doubles at regular intervals ranging from about 15 minutes to 1 hour. This means that if we start with 1,000 cells with a generation time of 30 min. then after an hour we end with 4,000 cells. In the next section of this module the parameters affecting bacterial growth will be discussed.

Bacteria are also known as prokaryotes because they don't possess nuclei; i.e., their chromosome is composed of a single closed double-stranded DNA circle. Structurally, a prokaryotic cell has three architectural regions: appendages (attachments to the cell surface) in the form of flagella and pili (or fimbriae); a cell envelope consisting of a capsule, cell wall and plasma or inner membrane; and a cytoplasmic region that contains the cell genome (DNA), ribosomes and various sorts of inclusions. Following is a brief discussion of these structural components.

- Cell envelope- is made of three layers: cytoplasmic membrane (inner layer), the cell wall (relatively rigid outer layer called peptidoglycan), and – in some bacterial species- an outer capsule. The role of the bacterial capsule is to keep the bacterium from drying, can serve as a virulence factor and as an antigen for identification, mediate adherence of cells to surface (crucial in biofilm formation), and confer protection against engulfment and attack by antimicrobial agents of plants, animals, and the environment. Bacteria can be placed into two basic groups, Gram-positive or Gram-negative, based on the profiles of the bacterial cell wall (see below).

- Chromosome- where the bacterium's genetic information is contained. It is a crucial tool for genetic fingerprinting (will be discussed further in this module).

- Cytoplasm- is where the function for cell growth, metabolism, and replication are carried out. It is composed of water, enzymes, nutrients, metabolic wastes, and gases; it also contains the ribosomes, chromosomes, and plasmids. As mentioned before, the cell envelope encases the cytoplasm and all its components.

- Flagella- are hair-like structures that serve as propellers to help bacterium move toward nutrients and away from toxic chemicals. This structure can be found at either or both ends or all over the bacterium surface and serve as antigen (H-antigen) for serotyping. Also, this organelle is a contributor for biofilm formation.

- Pili and fimbriae- many species of bacteria have these small hair-like projections emerging from the outside cell surface. Its function is to assist in attaching to other cells and surfaces. Specialized pili are used for passing nuclear material between bacterial cells (conjugation).

- Plasmid- short length of extra-chromosomal genetic structure (circles or loops) which are carried by many strains of bacteria. They are not involved in reproduction but replicate independently of the chromosome and are instrumental in the transmission of special properties, such as antibiotic drug resistance, resistance to heavy metals, and virulence factors necessary for infection of animal and human hosts. Plasmids are extremely useful tools in the area of genetic engineering.

- Ribosomes- these are organelles that translate the genetic code DNA to amino acids which are the building blocks of proteins. They are also an important tool in the fields of molecular biology and genetics.

- Spores- produced by some species and they are resistant to hostile conditions such as heat and drying. They serve as survival mechanisms when environmental conditions are not suitable for growth and replication.

The cell wall of bacteria is dynamic and extremely important for several reasons:

1. They are an essential structure for viability; protects the cell protoplast from mechanical damage and from osmotic rupture or lysis.

2. They are composed of unique components found nowhere else in nature.

3. They are one of the most important sites for attack by antibiotics.

4. They provide ligands for adherence and receptor sites for drugs or viruses.

5. They cause symptoms of disease in humans and animals.

6. They provide for immunological distinction and immunological variation among strains of bacteria.

7. They can be modified to protect the cell against harsh environmental conditions like heat, pH, antimicrobials, etc.

Cell wall composition varies widely amongst bacteria and is an important factor in bacterial species analyses and differentiation. The main functions are to give the cell its shape (rod, sphere, helix, or comma) and surround the cytoplasmic membrane, protecting it from the environment. As mentioned above, the profiles of the cell walls of bacteria, as seen with the electron microscope, make it possible to distinguish two basic types of bacteria as follows:

- Gram-positive bacteria (those that retain the purple crystal violet dye when subjected to the Gram-staining procedure) - the cell wall adjoining the inner or cytoplasmic membrane is thick (15-80 nanometers), consisting of several layers of peptidoglycan, also known as murein. Intertwine within the cell wall are polymers composed of glycerol, phosphates, and ribitol, know as teichoic acids. In general, Gram-positive bacteria produce extracellular substances that typically account for most of the virulence factors and this is illustrated by *Staphylococcus aureus*.

- Gram-negative bacteria (which do not retain the crystal violet) - the cell wall adjoining the inner membrane is relatively thin (10 nanometers) and is composed of a single layer of peptidoglycan surrounded by a membranous structure called the outer membrane. The outer membrane of Gram-negative bacteria invariably contains a unique component, lipopolysaccharide (LPS or endotoxin), which is toxic to animals. This outer membrane is usually thought of as part of the cell wall. The pathogenesis and virulence properties of Gram-negative bacteria are far more complex including outer membrane components as well as the production of extracellular substances which can be illustrated by *E. coli* O157:H7.

It may be advantageous for epidemiological purposes to identify a particular bacterial strain by serotyping, which is a useful tool to accomplish this goal. Previously we mentioned that there are components in the cell envelope that serves as antigens for serotyping, therefore, serotyping is based on the ability of the bacteria to agglutinate antibodies specific for those antigens. Following is a brief description regarding to the serotyping of those pathogens of public health concern.

Serotyping of Gram-negative bacteria (examples: *E. coli* and *Salmonella* spp.) consist of the immunoreactivity of three classes of antigens: the O-antigen (somatic), H-antigen (flagellar), and the K-antigen (capsular) surface profiles. The O-antigen is a polysaccharide which is a polymer of O-subunits, composed of 4-6 sugar residues, attached to the lipid A-core polysaccharide portion of the LPS molecule. Differences in the immunoreactivity of antibodies (O antiserum) with the O-antigen result from the variation in the sugar components and/or covalent linkages between the O-subunit. On the other hand, the H-antigen is the filamentous portion of the flagella which is composed of protein subunits called flagellin. The antigenically variable portion of flagellin determines the H serotype as determined by H antiserum. Finally, the K-antigens are the somatic or surface antigens that occur as envelopes, sheaths, or capsules. They act as masking antigens for the O-antigen, inhibiting agglutination of living cell suspensions in O antiserum (for the purpose of the scope of this module this antigen will not be further discussed). A specific combination of O- and H-antigens defines what is known as the serotype and/or serogroups of a bacterial isolate. The serotype and serogroups in particular species provide identifiable chromosomal markers that correlate with specific bacterial virulent clones. More than 2,500 *Salmonella* serotypes have been described and reported; examples are *S.* Enteritidis and *S.* Newport which belong to Serogroup D and B, respectively. In *E. coli*, a total of 170 different O-antigens and 55 H-antigens, defining the isolate serotype, have been identified; a well known example is *E. coli* O157:H7 serotype which is part of the enterohemorrhagic (EHEC) serogroup.

Likewise, the serotyping of Gram-positive bacteria (an example is *Listeria monocytogenes*) is based on the combination of somatic (O; teichoic acids) and flagellar (H) antigens. Although serological confirmation is not necessary for regulatory identification of *L. monocytogenes*, it is useful for determining the prevalence of specific

serotypes in epidemiological studies and for environmental recontamination tracking. Strains of *L. monocytogenes* can be assigned to 13 different serotypes, based on their combination of O- and H-antigens. While all of them are considered to be potentially pathogenic, most (>95%) human clinical isolates belong to three serotypes 1/2a, 1/2b, and 4b.

It is evident that bacteria are a complex system with the capability to adapt and survive to adverse environmental conditions. This explains, in part, why there are some microorganisms that are very difficult to eliminate (biofilm formation), why other becomes pathogenic, and why other develops resistance toward antibiotics or antimicrobial interventions. In slaughter as well as in the processing establishments there are bacterial species associated with particular meat and poultry products, including the environment.

## PARAMETERS AFFECTING THE GROWTH OF MICROORGANISMS

There are basically two parameters that affect the growth of microorganisms in food products, extrinsic and intrinsic. Extrinsic parameters are those properties of the environment (processing and storage) that exist outside of the food product which affect both the foods and their microorganisms. In the other hand, intrinsic parameters, are properties that exist as part of the food product itself, for example, tissues are an inherent part of the animal that may, under a set of conditions, promote microbiological growth.

Following is a list of these parameters that either may result in multiplication or inhibition of microbial growth in meat, poultry, or egg product.

Examples of intrinsic parameters are:

> pH: It has been well established that most microorganisms grow best at pH values around 7.0 (6.6 – 7.5), whereas few grow below a pH of 4.0. Bacteria tend to be more fastidious (complex nutritional or cultural requirements for growth) in their relationships to pH than molds and yeasts, with the pathogenic bacteria being the most fastidious. Most of the meats have a final pH of about 5.6 and above; this makes these products susceptible to bacteria as well as to mold and yeast spoilage.
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> Moisture content (water activity [$a_w$]): One of the oldest methods of preserving foods is drying or desiccation. The preservation of foods by drying is a direct consequence of removal or binding of moisture, without which microorganisms do not grow. It is now generally accepted that the water requirements of microorganisms should be described in terms of water activity ($a_w$) in the environment. Basically, the water molecules are loosely oriented in pure liquid water and can easily rearrange. When a solute is added (like salt) to water, the water molecules orient themselves on the surface of the solute, in this case the $Na^+$ and $Cl^-$ ions, and the properties of the solution change dramatically. Therefore, the microbial cell must compete with solute molecules for free water molecules. The water activity of pure water is 1.00; the addition of solute decreases $a_w$ to less than 1.00. Most foodborne pathogenic bacteria require $a_w$

greater than 0.9, however, *Staphylococcus aureus* may grow in $a_w$ as low as 0.86.

Oxidation-reduction potential: Microorganisms display varying degrees of sensitivity to the oxidation-reduction potential (O/R or EH) of their growth medium or environment. Aerobic microorganisms require more oxidized environments (more oxygen) versus anaerobic organisms which require more reduced environments (lacking oxygen).

Nutrient content: In order to grow and function normally, the microorganisms of concern in the food industry require the following: water, source of energy, source of nitrogen, vitamins and related growth factors, and minerals.

Antimicrobial constituents: The stability of some foods against attack by microorganisms is due to the presence of certain naturally occurring substances that have been shown to have antimicrobial activity. Nisin and other bacteriocins are good examples.

Biological structures: The natural covering of some food sources provides excellent protection against the entry and subsequent damage by spoilage organisms. Examples of such protective structure are the hide, skin and feathers of animals.

Examples of extrinsic parameters are:

Storage temperature: Microorganisms, individually and as group, grow over a wide range of temperatures. It is important to know the temperature growth ranges for organisms of importance in foods as an aid in selecting the proper temperature for product storage. A helpful reference is the FDA's Food Code (http://www.cfsan.fda.gov/~dms/fc05-toc.html); it contains some recommendations for storage temperatures of product that are widely accepted in the scientific community.

Relative humidity: The relative humidity of the storage environment is important both from the standpoint of water activity ($a_w$) within foods and the growth of microorganisms at the surfaces. Humidity can also be an important factor to consider when producing some types of product.

Presence/concentration of gases: Carbon dioxide ($CO_2$) is the single most important atmospheric gas that is used to control microorganisms in foods. It has been shown to be effective against a variety of microorganisms. Because of its effectiveness, $CO_2$ is used as one of the methods for modified-atmosphere packaging (refer to FDA Food Code).

Presence/activities of other microorganisms: The inhibitory effect of some members of the food microbiota on other microorganisms is well established. Some foodborne organisms produce substances that are either inhibitory or lethal to others. These include antibiotics, bacteriocins, hydrogen peroxide, and organic acids (such as lactic acid). General microbial interference is a phenomenon that refers to general nonspecific inhibition or destruction of one microorganism by other members of the same habitat or environment; the

mechanism for this interference is not very clear. Some of the possibilities are: competition for nutrients; competition for attachment/adhesion sites; unfavorable alteration of the environment and/or combinations of these.

## ISOLATION AND IDENTIFICATION OF PATHOGENS

You have learned during the FSRE Training that FSIS is responsible for aseptically collecting samples to determine the presence of pathogens (*E. coli* O157:H7 and *Listeria monocytogenes*) and *Salmonella* species according to the regulations. Once these samples are received by the Agency's laboratory, how are they processed?

When samples are received by any of the three field service laboratories (Athens, Ga.; St. Louis, Mo.; or Alameda, Calif.) it is first subject to a selective enrichment procedure, to favor growth of the desired organism, followed by an initial screening test for presumptive positives. The BAX® system is used as one of the initial screening test for the detection of *Salmonella, Listeria monocytogenes* and *Escherichia coli* O157:H7, and is based on the polymerase-chain reaction (PCR) technology which has proven to be rapid and highly sensitive. Thereafter, those found to be a screening positive will be further confirmed using immunological, biochemical, and molecular methods.

Let's look at an example pertaining to the isolation and characterization of *E. coli* O157:H7 and/or O157:H7/NM from raw and ready-to-eat beef products. The first step is to enrich the samples using an enrichment broth suitable for this pathogen followed by the screening test using the BAX® system in conjunction with alternative (lateral flow devices) screening test. Those samples found to be positive (potential positive) are further processed by performing an immuno-magnetic separation using magnetic beads coated with O157-antibodies and plating an aliquot on a selective media (Rainbow agar); plates are then incubated for 18-24 hours at 35˚C. One or more typical colonies are tested with O157 antiserum and colonies that show agglutination (presumptive positives) are processed for confirmation by performing serological, biochemical, Shiga toxin assays, and genetic analyses. The time frame for reporting potential positive or screen negative result is two days; presumptive positive is 3 days; and confirm positives is 5-7 days. Please note that the example discussed above as well as the other two microorganisms (see below) does not include follow-up testing (e.g., NVSL serotyping, PFGE fingerprinting) and the days listed do not include delays (e.g., re-streak for purity).

The isolation and characterization of *L. monocytogenes* and *Salmonella* spp follows the same rationale as discussed in the previous example using the appropriate culture media and assays. The time frame for reporting the test results of these microorganisms is as follows:

- *L. monocytogenes:* for screen negative is 3 days; presumptive positive is 4-5 days (a sample from which one or more typical colonies produces beta-hemolysis on Horse Blood agar); and confirmed positive is 5-8 days (when a beta-hemolytic isolate is CAMP test positive, shows tumbling motility, and is characterized biochemically).

- *Salmonella* spp: for screen negative is 2 days; presumptive positive is 5 days (when a sample yields one or more isolates which show typical appearance on

TSI and LIA slants, and agglutinate *Salmonella* somatic antisera); and confirmed positive is 7 days (*Salmonella* O group positive isolates are characterized biochemically as the genus).

These results are then posted on LEARN to be accessible for the FSIS inspection personnel. Remember that, in the case of *E. coli* O157:H7 and *L. monocytogenes* in ready-to-eat (RTE) products, presumptive positives reports are also posted so immediate action can be taken by the establishment concerning to the adulterated product.

## PRIMARY SOURCES OF MICROORGANISMS IN FOOD

From the meat and poultry regulatory perspective, we will be addressing bacteria as a main source of food contamination. Keep in mind that there are other microorganisms like viruses, parasites, fungi, etc., that are able to contaminate food and cause foodborne illnesses in animals and humans.

Bacteria can be found virtually everywhere including humans and can enter food products through different routes. The following list outlines some of the most common ways in which microorganisms enter food products.

Soil, water, and in-plant environment: Many bacteria are carried in soil and water which may contaminate food. Also the in-plant environment is an important source of contamination because of the daily activities and pest infestation. *Listeria, Clostridium, Salmonella,* and *Escherichia* are good examples.

Animal feeds: This is a source of salmonellae to poultry and other farm animals. It is a known source of *Listeria monocytogenes* to dairy and meat animals when fed silage. The organisms in dry animal feed are spread throughout the animal environment and may be expected to occur on animal hides, hair, feathers, etc.

Animal hides: The hide is a source of bacterial contamination of the general environment, hands of establishment employees, and skinned carcasses. Studies have shown that this may be a primary source for *E. coli* O157:H7, *Salmonella*, and *Listeria* in cattle.

Gastrointestinal tract: The intestinal biota consists of many organisms; notable among these are pathogens such as *Salmonella, Campylobacter, E. coli* O157:H7, and other microorganisms. Any or all of the Enterobacteriaceae may be expected in feces of livestock and poultry.

Food handlers: The microbiota on the hands and outer garments of handlers generally reflect the environment and habits of individuals (hygiene), and the organisms in question may be those from hides, gastrointestinal tracts, soil, water, dust, and other environmental sources.

Food Utensils: Saws, cutting boards, knives, grinders, mixers, etc. may become contaminated during slaughter and processing operations and ensure a fairly constant level of contamination of meat-borne organisms.

Air and dust: A variety of bacteria may be found in air and dust in food-processing operations at any one time. *Listeria* is an example of a Gram-positive organism that survives in the environment.

Vegetables (plant) and vegetable products: May be a significant concern in the processing of meat, poultry and egg products. A good example is the processing of frozen entrees, salads, etc. containing meat and poultry components. Many or most soil and water organisms contaminate vegetables and fruits.

Globalization of food supply: This is a major factor of contamination resulting in transfer of pathogenic agents between countries (import/export) such as Bovine Spongiform Encephalopathy (BSE) infective agent and *Salmonella* Typhimurium DT104, among others. Also, with the increase in international travel this imposes a risk of introducing pathogens to this country like Foot and Mouth Disease.

Terrorist attacks: There are growing concern in the food industry that terrorist could use pathogens to contaminate food and water supplies in attempt to disrupt the economy, health, and lifestyle among others.

## HOW DOES FOOD BECOME CONTAMINATED?

We live in a microbial world, and there are many opportunities for food to become contaminated as it is produced and prepared. Many foodborne microbes are present in healthy animals (usually in their intestines, hides, feathers, etc) raised for food. Meat and poultry carcasses can become contaminated during slaughter by contact with small amounts of intestinal contents or poor dressing procedures. Also, it has been shown scientifically that some *Salmonella* serotypes can infect a hen's ovary in such a manner that the internal contents of a normal looking egg can be contaminated with *Salmonella* even before the shell is formed.

In food processing, foodborne microbes can be introduced from infected humans who handle the food, or by cross contamination from some other raw agricultural product and/or the in-plant environment. For example, bacteria and viruses can be introduced by the unwashed hands of food handlers who are themselves infected.

In the RTE processing environment exposed product that is fully cooked can become cross contaminated if it touches raw meat or poultry that contain pathogens or from food contact surfaces that are contaminated.

In the kitchen, microbes can be transferred from one food to another food by using the same knife, cutting board or other utensil to prepare both without washing the surface or utensil in between.

The way that food is handled after it is contaminated can also make a difference in whether or not an outbreak occurs. Many microorganisms need to multiply to a larger number before enough are present in food to cause disease. Given warm moist conditions and an ample supply of nutrients, one bacterium that reproduces by dividing itself every half hour can produce 17 million progeny in 12 hours. As a result, lightly

contaminated food left out overnight can be highly infectious by the next day. If the food were refrigerated promptly, the bacteria would not multiply at all or at a very slow rate.

To inhibit bacterial growth in meat, poultry, or egg products or in food handled by the consumer, it is important to store foods at a reduced temperature. Refrigeration or freezing prevents virtually all bacteria from growing but freezing preserves them in a state of suspended animation.

## FOODBORNE ILLNESS

Microorganisms can cause a variety of effects in food products including spoilage, which primarily affects product quality, and food poisoning, which is generally caused by pathogens. As regulators, we are most concerned with the effects that microorganisms have on food that leads to foodborne illness, because this affects public health.

A foodborne illness (or disease) is exactly what the term indicates - a disease or illness caused by the consumption of contaminated foods or beverages. It would seem rather obvious that a foodborne microbial pathogen, or a preformed microbial toxic product, or another poison such as a poisonous chemical that has somehow contaminated the food and/or beverage, leads to one of the many different foodborne illnesses.

There is no one “syndrome” that is representative of foodborne illness/disease. Different diseases have many different symptoms. However, the microbe or toxin enters the body through the gastrointestinal tract, and often causes the first clinical signs such as nausea, vomiting, abdominal cramps and diarrhea which are common symptoms in many foodborne diseases.

More than 250 different foodborne diseases have been described. Most of these diseases are infections, caused by a variety of bacteria, viruses, and parasites. Other diseases are poisonings, caused by harmful toxins or chemicals that have contaminated the food, for example, poisonous mushrooms or heavy metal contamination.

There are several hurdles that the pathogen must overcome in order to cause illness. A simple summary of these hurdles are as follows.

-Survive the acidic environment of the stomach.
-Attach to/colonize intestinal walls.
-Compete against the natural microbiota of the gut.
-Survive the host defense mechanisms.
-Once attached in the large intestine: elaborate toxins and virulence factors, and cross the epithelial barrier, which then results in the symptoms characteristic to the disease or illness.

## FOODBORNE PATHOGENS

Following is a list of pathogens and infectious agents of public health concern. This list is not exhaustive; however, it contains most of the foodborne pathogens that affect meat, poultry, and egg products.

1. Bacteria

   Gram Positive:
   -*Listeria monocytogenes*
   -*Staphylococcus aureus*
   -*Bacillus cereus*
   -*B. anthracis*
   -*Clostridium botulinum*
   -*C. perfringens*

   Gram Negative:
   -*Salmonella* spp
   -*Campylobacter* spp
   -*Escherichia coli* 0157:H7
   -*Yersinia enterocolitica*
   -*Brucella* spp

2. Viruses:
   -Hepatitis
   -Rotaviruses

3. Prions:
   -new variant CJD

4. Tapeworms:
   -*Taenia* spp

5. Roundworms:
   -*Trichinella* spp

6. Protozoa:
   -*Toxoplasma* spp
   -*Sarcocystis* spp

The Centers for Disease Control (CDC) reports that the most commonly accounted foodborne infections are those caused by the bacteria *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, and *E. coli* O157:H7; and by a group of viruses called calicivirus, also known as the *Norwalk* and Norwalk-like viruses ("http://www.cdc.gov/ncidod/dbmd/diseaseinfo/default.htm" ). We will be discussing these aforementioned microorganisms because they are of concern to the food industry, to FSIS as a public health regulatory agency, and the consumer.

**Pathogens and Infectious Agents of Concern from the Public Health Regulatory Perspective**

***Salmonella* spp**

*Salmonella* is a rod-shaped, motile bacterium (non-motile exceptions are *S.* Gallinarum and *S.* Pullorum), non-spore forming and Gram negative. This microorganism grows at 6.5- 47˚C (43.7-116˚F), pH as low as 4.5, with or without air, and $a_w$ of >0.95 (may vary, e.g., *S.* Newport = 0.941 and *S.* Typhimurium = 0.945). The optimum growth

temperature is at the human body temperature but it grows very poorly at refrigerated temperatures. Even though freezing and frozen storage can have some deleterious effect on *Salmonella* it is known that this microorganism remains viable for long periods of time in frozen foods. There are specific serotypes that are capable of producing foodborne illness (salmonellosis) including *S.* Enteritidis (eggs and egg products), *S*. Newport (milk and dairy cows), and *S.* Typhimurium (cattle) among others.

*Salmonella* spp. have the ability to cross the mucosal barrier invading and replicating within the host causing chronic infections, long term carriage, and systemic disease. Pathogenic *Salmonella* possess a myriad of virulence factors including those that promote adhesion to host cells in the intestine, endotoxins, siderophores, invasins, and the production of cytotoxins and diarrheagenic enterotoxins, which act in concert in the pathogenesis of infection. It is believed that the enterotoxins are responsible in causing the acute symptoms of the disease.

As of 2002 there were 2,541 *Salmonella* serotypes identified and approximately 2,000 serotypes cause human disease. The CDC has estimated 1.4 million cases occur annually in the United States but approximately 2.14% (culture-confirmed) of those cases are reported to CDC. Also, annual estimates of over 500 cases are fatal and 2% of the salmonellosis cases are complicated by chronic arthritis. Furthermore, salmonellosis is more common in the summer than winter. In 2005, a total of 36,184 isolates were reported from participating public health laboratories which represents 1 1.5%increase compared with 2004. The national rate of reported *Salmonella* isolates (2005) was 12.2/10,000 people. From that total, the five most frequently reported *Salmonella* serotypes from human sources (expressed in per cent) to CDC encompass *S.* Typhimurium (includes var. 5-)(19.3%), *S.* Enteritidis (18.6%), *S.* Newport (9.1%), *S.* Heidelberg (5.3%), and *S.* Javiana (3.7%). (PHLIS Surveillance Data, Salmonella at http://www.cdc.gov/ncidod/dbmd/phlisdata/salmonella.htm). The four most common aforementioned serotypes in 2005 (have been in this order since 1995) represent 52% of all isolates.

The cumulative (year-to date) of salmonellosis cases reported by CDC for the years 2006 and 2007 were 35,561 and 34,180, respectively. All age groups are susceptible to salmonellosis, but symptoms are most severe in the elderly, infants (<5 yrs), and those individuals with impaired immune systems. AIDS patients suffer salmonellosis frequently (estimated 20-fold more than general population) and suffer from recurrent episodes. This microorganism is usually transmitted to humans by ingestion of contaminated foods of animal origin, such as beef, poultry, milk, or eggs. As mention before, the organism penetrates and passes from the gut lumen into the epithelium of small intestine where inflammation occurs. The enterotoxins produced by *Salmonella*, perhaps within the enterocyte, cause acute symptoms such as nausea, vomiting, abdominal cramps, diarrhea, fever, and headache. Chronic consequences can include arthritic symptoms which may follow 3-4 weeks after onset of acute symptoms. The onset time of the disease typically ranges from 6-48 hours and the minimal infective dose (MID; is the minimum number of pathogenic cells require to cause infection) can result from as few as 15-20 cells; the symptoms may last from 1 to 7 days or may be prolonged depending upon age, health of host, ingested dose, and the degree of pathogenicity (virulence) among the members of the genus.

Data on *Salmonella* isolates obtained from non-human sources (animals, feed, and environment) can help identify possible sources of human illness. The four most

common serotypes of *Salmonella* isolated in livestock and poultry in 2003 and 2004 are *S.* Enteritidis (Serogroup D), *S.* Typhimurium (Serogroup $C_2$), *S.* Newport (Serogroup B), and *S.* Heidelberg (Serogroup $C_2$) which accounted for approximately 50% of the isolates reported to CDC.

The epidemiology of *Salmonella,* based on serotype characterization, has been changing; *S. enterica* serotype Typhimurium has decreased in incidence while the incidence of serotypes Newport, Mississippi and Javiana have increased. Following we are going to discuss in more details the five common serotypes that causes illness in humans.

*Salmonella* Typhimurium, the most common serotype in humans, is identified from clinical samples (results from clinical of animal disease) from bovine and porcine sources, and from non-clinical samples (results from animal surveillance and food products) from chicken sources. Outbreaks of *S.* Typhimurium infections have been associated with the consumption of ground beef. Rates of antibiotic resistance among certain serotypes have been increasing; a substantial proportion of serotypes Typhimurium and Newport isolates are resistant to multiple drugs. A large portion of the isolates recovered from humans were resistant to multiple antimicrobial drugs including those with a five-drug resistant pattern characteristic of the *S.* Typhimurium phage type DT104 (26% in 2003). Recently, Davis *et al* (2007, Emerging Infectious Diseases, Vol. 13, 1583-1586; www.cdc.goc/eid) compared the antimicrobial-drug resistance profiles and PFGE profiles of human and bovine *S.* Typhimurium isolates (2002-2006; strains TYP035/TYP 187) originated from the Pacific Northwest. They concluded that these strains may represent an emerging epidemic clonal strain in this region of the United States.

*Salmonella* Enteritidis (SE), the second most common serotype in humans, are identified from clinical and non-clinical chicken sources. The present situation with SE is complicated by the presence of the organism inside the egg, in the yolk. This and other information strongly suggest vertical transmission, i.e., deposition of the organism in the yolk by an infected layer hen prior to shell deposition. Specific control programs (e.g., farm-based egg-quality assurance programs) have led to the reduction of SE infections, which have been associated with the consumption of internally contaminated eggs Foods other than eggs have also caused outbreaks due to SE.

While the number of human infections caused by the previous top two serotypes had substantial decreases from 1994-2005, *Salmonella* Newport has emerged as a major multidrug-resistant pathogen (resistant to at least nine of 17 antimicrobial agents tested), becoming the third most common serotype in the United States. This serotype has been identified from clinical bovine sources. Between 2002 and 2004 CDC reported four outbreaks of antimicrobial resistant *Salmonella* infection that implicated FSIS regulated products, including three attributed to ground beef. Two of the three ground beef associated outbreaks were linked with *S*. Newport infection. Lastly, *S.* Heidelberg was the fourth most common serotype in humans in 2003 and 2005, and the fifth most common in 2004; is identified from clinical porcine sources as well as clinical/non-clinical chicken and turkey sources.

The prevalence of the pathogen *Salmonella* in beef, lamb, pork, and poultry carcasses varies greatly. The overall contamination of meat and poultry carcasses with these pathogens depends not only on the numbers of the pathogens on the hair, hide,

feathers, skin, and in the intestinal tract of the animals, but is also significantly affected by the degree of cross-contamination occurring from these sources during slaughter and processing.

Also, *Salmonella* has been isolated from milk and dairy products, fish, shrimp, frog legs, yeast, coconut, sauces and salad dressing, cake mixes, cream-filled desserts and toppings, dried gelatin, peanut butter, cocoa and chocolate, etc.

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

The establishments that slaughters and/or process meat and poultry products must adhere to pathogen reduction performance standards for *Salmonella*, as specified in 9 CFR 310.25 for livestock and in 9 CFR 381.94 for poultry. Between 2002 and 2005, USDA reported an increase in the in the percentage of chicken carcasses that tested positive for *Salmonella*, from 11.5 to 16.3%.; including a significant increase in SE. On February 27, 2006 (Federal Register, Docket No. -4-026N), FSIS posted a new approach to *Salmonella* verification activities in meat and poultry establishments as follows:

- Report each *Salmonella* test result to the establishment as soon as it is available.
- Post quarterly nationwide *Salmonella* data to the FSIS website for each product class.
- FSIS is collecting samples in establishments slaughtering young turkeys.
- Classify inspected establishments into *Salmonella* categories based on process control. The Agency will allocate more laboratory resources to establishments with marginal *Salmonella* control.
- Assess food safety systems in establishments with poor and variable *Salmonella* control.
- Published new guidelines for *Salmonella* control in chicken slaughter establishments.
- Rapidly share serotype information with establishments. The identified serotypes isolated from the establishments will be compared to the CDC list of the20 most frequently *Salmonella* serotypes that causes illness in humans.
- Use other subtyping data in the new *Salmonella* program.
- Conduct on-going studies to monitor pathogen control in raw product. FSIS will conduct additional baseline studies to measure the national prevalence of *Salmonella* on raw products.
- Track progress in *Salmonella* control for all tested product classes.

In a short period of time the FSIS has also developed guidelines and procedures for the comprehensive assessment of food safety systems in poultry establishments with less than consistent *Salmonella* process control. Furthermore, the Agency has accomplished a new risk-based approach allocating *Salmonella* sampling resources. Since April 2006, FSIS has been providing these results; this quarterly report can be access on FSIS Web site at http://www.fsis.usda.gov/Science/Q2_2006_Salmonella_Testing/index.asp.

## ***Escherichia coli* O157:H7**

A minority of *E. coli* serotypes are capable of causing human illness (colibacillosis) by different mechanisms. Naturally *E. coli* is a normal inhabitant of the intestine of all animals, including humans; serves a useful function in the body by suppressing the growth of harmful bacterial species and by synthesizing appreciable amounts of vitamins.

Based on disease syndromes and other characteristics, there are six classes of diarrheagenic *E. coli* recognized: enteroaggregative (EAggEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enterohemorrhagic (EHEC), and diffusely adherent (DAEC). EHEC is the class that is of concern to industry, FSIS, and public health; the more significant serotype is *E. coli* O157:H7.

*Escherichia coli* serotype O157:H7 is one of the rare serotype of this genus and, as mentioned above, belongs to the EHEC family that causes severe disease. This pathogen is a rod-shaped, generally motile, non-spore forming and Gram-negative. It generally grows at 2.5-45˚C (36.5-113˚F), pH between 4.6-9.5, with or without air, and $a_w$ of >0.935. There are strains of *E. coli* O157:H7 that possess an unusual tolerance to environmental stress such as temperature, pH, dryness, and can survive in water; recent research have shown that some strains are capable of forming biofilms.

This pathogen produces several virulence factors that cause severe damage to the lining of the intestine, acute renal failure (children and elderly), hemolysis, thrombocytopenia, and neurological problems (the last three occur mainly in adults). All EHEC, including *E. coli* O157:H7, produce Shiga toxins (Stx 1 and 2; also known as Vero toxins and Shiga-like toxins) which are closely related to or identical to the toxin produced by *Shigella dysenteriae* type 1; these toxins targets the human kidney, particularly the cortical region which is rich in $Gb_3$ receptors for the toxin. These toxins are encoded on a bacteriophage that was transferred from *Shigella* to *E. coli* O55:H7 (parent strain of serotype O157:H7). Other virulence factors are the pO157 plasmid (90-kb size) which encodes the EHEC hemolysins and serine proteases; LEE pathogenic island which enclose the genes accountable for the A/E histopathology including a type III secretion system responsible for the epithelial cell signal transduction events leading to the attaching/effacing (A/E) lesion, and a bacterial adhesion proteins called intimin and Tir (Translocated intimin receptor); as well as other virulence factors.

Data collected by CDC through the National Notifiable Diseases Surveillance System (NNDSS) in collaboration with the Council of State and Territorial Epidemiologists (CSTE) have shown that during 1996-2004, the estimated cases of infections with *E. coli* O157:H7 had a substantial decline (2005, MMWR 54(14):352-356). During 2004 through 2005 (cumulative, year-to-date) a total of 2544 and 2461 cases of EHEC O157:H7, respectively, have been reported. *Escherichia coli* O157:H7 has been nationally notified since 1994. Surveillance categories for EHEC infection include EHEC O157:H7, serogroup non-O157, and EHEC not serogroup. During 2005, cases of EHEC infection were reported from 50 states, the District of Columbia, and Puerto Rico. Of these, 74% were classified as EHEC O157:H7; 14% as EHEC, serogroup non-O157; and 12% as EHEC, not serogroup. The majority of cases were reported during July-October.

Interestingly, the Shiga-toxin positive, serogroup non-O157 EHEC are on the rise with a total of 242 and 266 reported cases corresponding to 2003 and 2004 (cumulative, year-to-date), respectively. Since 2005, Morbidity and Mortality Weekly Report (MMWR, CDC) has been reporting the data as Shiga-toxin-producing *E. coli* (STEC) which includes O157:H7, serogroup non-O157, and not serogroup making it difficult to assess the predominance of each individual STEC. As of 2006 through October 26, 2007 (Cumulative, year-to-date) there has been 3,359 and 3,518 cases reported, respectively. This has prompted FSIS, in conjunction with other Federal Agencies, to hold a public meeting (Federal Register Docket FSIS-2007-0041, Oct 9, 2007) to consider the public health significance of STEC non-O157. The scientific community believes that STECs that are pathogenic not only contain the Shiga toxin but also additional virulence determinants that, together with the toxin, causes illness similar to those caused by *E. coli* O157:H7. It is widely accepted that the prevalence of STEC non-O157 be underrepresented due to the limitations of the protocols (can vary and are not implemented uniformly) for the isolation of non-O157 enteric pathogens in clinical laboratories. The O-antigen that has been identified to a large number of the non-O157:H7 isolates include O26, O111, O103, O121 and O145.

This microorganism causes three distinctive clinical manifestation including hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP). All people are believed to be susceptible to hemorrhagic colitis, but young children and the elderly appear to progress to more serious symptoms more frequently (HUS and TTP, respectively).

HC is characterized by severe cramping (abdominal pain) and diarrhea which is initially watery but becomes grossly bloody. Occasionally vomiting occurs and some individuals can exhibit watery diarrhea only. Fever is either low-grade or absent. The infectious dose is as few as 10 bacterial cells with an incubation period of approximately 4 days (median) and clinical manifestations can develop within 24-48 hours with duration of 8 days (average).

A week after the onset of gastrointestinal symptoms with this pathogen some victims (particularly the very young under the age of 10) have developed HUS, characterized by the triad hemolytic anemia, thrombocytopenia, and renal failure. Permanent loss of kidney function may result and the mortality rate in children is 3-5%. During 2005, the majority of reported cases occurred among children aged <5 yrs. In addition, the total HUS (postdiarrheal) cases reported from the last 3 years has shown an increase, i.e., from 2005-2007 there has been 221 cases (2005), 288 cases (2006) reported and as of October 26, 2007 only 171 cumulative cases has been reported which can be even higher.

In the adults and elderly, a complication associated with this microorganism is TTP characterized by central nervous system deterioration, seizures, and strokes. This illness can have a mortality rate in the elderly as high as 50%.

*Escherichia coli* O157:H7 is a bacterial pathogen that has a reservoir mainly in cattle; other reservoirs have been identified including pigs, sheep, flies, deer and other wild animals. In recent scientific studies, it has been shown that feedlot steers and heifers appear to carry the organism at higher levels than once thought, even higher than dairy cattle and calves. Also, it has been shown that *E. coli* O157:H7 is seasonal (April through September) peaking during summer.

Undercooked or raw hamburger (ground beef) has been implicated in many of the documented outbreaks. Because of its public health significance, the vast scientific evidence showing the high incidence in cattle, the severity of the illness, and outbreaks due to this pathogen, these events prompted FSIS (1994) to declare *E. coli* O157:H7 as an adulterant in meat (beef) products. By the year 2002, the Agency required all establishments producing raw beef to reassess their HACCP plans to determine if *E. coli* O157:H7 is a food safety hazard reasonably likely to occur in their production process (Fed Reg. Vol.67, No.194:62325-62334, October 7, 2002/Rules and Regulations). In 2005 FSIS published a notice (Fed Reg. Vol. 70, No. 101:30331-30334, May 26, 2005/Rules and Regulations) informing the establishments that produce mechanically tenderized beef products, including those that are injected with marinade, to reassess their HACCP plan by the year 2006. This reassessment was triggered by the fact that there have been three *E. coli* O157:H7 outbreaks associated with consumption of mechanically tenderized beef.

In June 2007, FSIS noticed an increased number of positive Agency *E. coli* O157:H7 results that occurred within a short period of time. As a result, FSIS decided to increase the number of scheduled raw ground beef product samples for testing during the month of July 2007 (FSIS Notice 41-07). However, in September 2007, there was a recall of 21.7 million pounds of frozen hamburger, the second largest recalls in US history, linked to 40 reported illnesses from a multi-state outbreak with 21 known hospitalizations. DNA fingerprint patterns were traced back from beef trim supplied by a foreign country firm. Thereafter, another recall of approximately 1.9 million pounds took place during the period of October-November 2007. So far in 2007 (Jan-Oct) there has been 15 recalls, in which 8 recalls were linked to illnesses, with a total amount of 29 million pounds of ground beef.

FSIS announced new, ongoing and upcoming actions to protect public health against the risk of *E. coli* O157:H7. Among the measurements to target *E. coli* contamination and adulteration of ground beef products, FSIS have issued a series of notices providing the inspection program personnel the instructions necessary to fully implement risk-based verification activities including:

- Collect samples of beef manufacturing trimmings and other raw ground beef and patty components for expanded testing including imported beef trim (FSIS Notices 17-07, 18-07, and 68-07).
- Notify countries that export beef to the USA about new policies and programs to control *E. coli* O157:H7 (FSIS News & Events 11/03/07- Statement from Dr. R. Raymond)
- Submit the raw beef sample collected to the laboratory after the establishment has completed all interventions and without waiting for the establishment to complete pre-shipment review (FSIS Notice 62-07).
- Requirement of establishments that produces raw beef products to verify that they are controlling *E. coli* O157:H7 by reassessing their hazard analysis and HACCP system, and provide processors with specific examples of controls that meet its safety criteria (Notice 65-07).
- Instructions to inspection program personnel at official establishments that slaughter, fabricate, grind, mechanically tenderize or enhance to complete an on-line checklist on how the establishment addresses *E. coli* O157:H7. The

information captured in this checklist will be used for targeted approaches for the risk-based verification program and to assist in prioritizing the scheduling of Food Safety Assessment (Notices 64-07 and 65-07).

- New instructions for multiple follow-up samples of raw ground beef, raw ground beef trimmings, and other raw beef patty components in response to an FSIS positive *E. coli* O157:H7 result or another Federal or State entity's positive *E. coli* O157:H7 result (Notices 17-07 and 66-07).
- The Agency is conducting outreach and training sessions for small and very small producers starting in November 2007.

Recently, on November 1, 2007, 3.3 million pounds of frozen meat pizza products were recalled. The problem was discovered following an investigation carried out by the Tennessee Department of Health in coordination with CDC into a multi-state cluster of 21 *E. coli* O157:H7 illnesses that may be linked to this product.

Additionally, *E. coli* O157:H7 outbreaks have also been implicated with alfalfa sprouts, unpasteurized fruit juices, dry-cured salami, spinach, lettuce, game meat, cheese curds, among others.

Other vehicles of infection with *E. coli* O157:H7 include person-to person transmission (child day care facilities), water (recreational, well, and municipal water systems), animal contact (farms and petting zoos), and diagnostic laboratory related.

***Listeria monocytogenes***

*Listeria* species (spp) is a rod-shaped, non-spore forming Gram-positive bacterium. Within the *Listeria* genus six species has been identified consisting of *L. monocytogenes, L. innocua, L. ivanovii, L. welshimeri, L. seeligeri,* and *L. grayi*. Specifically, *L. monocytogenes* is recognized as a human pathogen that causes listeriosis. This pathogen is motile and can grow in cool (temperature range of 0-45˚C [32-113˚F]) and damp environments, at a pH range of 4.4-9.4, and $a_w$ >0.92. Some characteristics that make some strains of *L. monocytogenes* hearty include growth and/or survival in acidic environment (pH < 5.0), ability to withstand heat treatments, and growth and/or survival in concentrated salt solutions. The pathogenicity of this microorganism is associated with the virulence factors such as internalin A (allow the pathogen to induce its own uptake by specific host cells), Act A (a surface protein required for intracellular movement and cell-to-cell spread through bacterially induced acting polymerization), listeriolysin O (a toxin that acts as a hemolysin), among others. Only three *L. monocytogenes* serotypes (4b, 1/2a, and 1/2b) are pathogenic and account for the majority of human infections in the United States.

One outstanding characteristic of *L. monocytogenes* is its ability of forming biofilm which serve as a protection shell. This pathogen as well as other biofilm microorganisms, elicits specific mechanisms for initial attachment (by the production of extra polymeric substance and the bacterial cell surface structures such flagella, fimbriae, and other proteins) to a surface, development of a community structure and ecosystem (biofilm), and detachment. Biofilm is a heterogeneous structure of microbial cells (can be a mix culture) encased in an extracellular polymeric substance matrix (primarily polysaccharide material) where non cellular material can be entrapped such as mineral crystals, corrosion particles, blood components, food particles, etc. Active flow occurs in this

nitch allowing diffusion of nutrients, water, oxygen, and even antimicrobial agents; there is also exchange of waste metabolic material. Since this ecosystem is dynamic, the community structure changes from a compact to a looser structure over time allowing the dispersion of planktonic cells to other sites and start the cycle of biofilm formation. Biofilm can form as little as a few hours to days depending of the number of bacterial cells, nutrient availability, surface characteristics, temperature, etc. Once formed they can persist for a long period of time (years), they are very difficult to remove, and it confer protection from the chemicals used to clean and sanitize surfaces.

The occurrence of listeriosis in the United States for the last three years is as follows: a total of 696 cases in 2003, 750 cumulative cases in 2004, and 798 cumulative cases in 2005 (the last two entries are based on preliminary data reported by CDC). Although the incidence of listeriosis decreased from the period of 1996-1998 through 2001, thereafter the incidence has been increasing in the last three years.

Generally, listeriosis occurs among the elderly, pregnant women (the outcome upon exposure of the fetus to the pathogen results in abortion, stillbirth, or neonatal sepsis), diabetics, those on kidney dialysis, and the immunocompromised (bone marrow transplant patients, corticosteroids and graft suppression, cancer patients- leukemic patients particularly, individuals with AIDS, etc.). Some reports suggest that normal, healthy people are at risk, although antacids or cimetidine may predispose.

Listeriosis in adults is characterized by two forms of the illness, an invasive form and noninvasive form. The noninvasive form is characterized by febrile gastroenteritis and it has been documented in several outbreaks. The onset time may be greater than 12 hours. In the invasive form, the manifestation of listeriosis include septicemia (mortality rate as high as 50%), meningitis (mortality rate as high as 70%), encephalitis, and intrauterine or cervical infections in pregnant women (mortality rate from perinatal/neonatal infections greater than 80%). The onset of the aforementioned disorders is usually preceded by influenza-like symptoms including persistent fever. The onset time to gastrointestinal symptoms is unknown but is probably greater than 18-20 hours and for the serious forms of listeriosis (invasive) is also unknown, may range from a few days to three weeks.

The infective dose of *L. monocytogenes* is unknown, however, may vary with the strain and susceptibility of the individual. From cases contracted through raw or pasteurized milk, it can be assumed that in susceptible persons, fewer than 1000 total organisms may cause disease. As an example, a listeriosis outbreak in Switzerland involving cheese suggested that healthy uncompromised individuals could develop the disease, particularly if the foodstuff was heavily contaminated with the organism. Summarizing, the risk of developing the disease will depend on the susceptibility of the individual, the bacterial strain (infectivity), ingested dose, and whether the food consumed is a high- or low-risk foods. Most healthy persons probably show no symptoms by consuming contaminated foods and some studies suggest that 1-10% of humans may be intestinal carriers of *L. monocytogenes*.

There are particular meat and poultry high-risk foods that are associated with listeriosis because of the potential for contamination, they support the growth (temperatures as low as 3˚ C [37.4˚F]) of *L. monocytogenes*, and the common denominator is that they are ready-to-eat (RTE). RTE products usually require refrigeration and are store for an

extended period of time. Examples of high-risk foods are hot dogs, deli meats, pâté and meat spreads.

Other foods that are associated with contamination by *L. monocytogenes* include raw milk, supposedly pasteurized fluid milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw meat sausages, raw poultry and meat (all types), and raw and smoked fish. As mentioned previously, the ability of this pathogen to grow at low temperatures permits the multiplication in refrigerated foods.

This pathogen has also been found in the gastro-intestinal tract of 37 mammalian species, both domestic and feral, as well as at least 17 species of birds and possibly some species of fish and shellfish. *Listeria* is ubiquitous in nature; it can be isolated from soil, silage, water, vegetation, and other environmental sources.

The pathogen *L. monocytogenes* is also ubiquitous in the plant environment (equipment, utensils, humans, water, air flow, etc) and its presence in the RTE environment can poise a serious problem, specially, in the RTE finished product and food contact surfaces. Product flow must be designed to segregate finished from raw products as well as restrictions of personnel who handles RTE products to prevent cross-contamination. The main concern in the RTE environment is the ability of *L. monocytogenes* to form biofilm which confer the microorganism to survive under adverse conditions such as freezing, drying, high salinity, antimicrobials, and heat. Thus, sanitation scrutiny including microbial analysis must be part of the establishment's quality control practice to avoid cross-contamination of the product and food contact surfaces.

Under the FMIA and PPIA, RTE product is adulterated if it contains *L. monocytogenes* or if the product comes into direct contact with a food contact surface that is contaminated with the microorganism. Government agencies (USDA, FDA) and the food industries have taken steps to reduce contamination of food by the *Listeria* bacterium. In 2003, USDA issued new regulations aimed at further reducing *L. monocytogenes* contamination of RTE meat and poultry products (USDA/FSIS: "Control of *Listeria monocytogenes* in ready-to-eat meat and poultry products"; Vol 68, p.34208-34254). FSIS requires the establishments producing RTE meat and poultry products to address control measures in their HACCP plans or to prevent contamination through their SSOP and/or pre-requisite programs. When a processed food is found to be contaminated, food monitoring and plant inspection are intensified, and if necessary, the implicated food is recalled.

**Bovine Spongiform Encephalopathy (BSE)**

Bovine Spongiform Encephalopathy (BSE) or "mad cow disease" is a progressive neurological disorder of cattle that results from infection by an agent known as a **pro**teinaceous **in**fectious particle or **prion** protein. This agent exists in two forms, namely, the normal ($PrP^{c}$) and its pathological isoform ($PrP^{res}$ ). The $PrP^{res}$ isoform is an abnormal shaped protein ($\beta$-pleated sheet) which lacks nucleic acids, resists protease digestion, and survives dry heat at 600˚C for 15 min. The normal isoform ($\alpha$-helix) is expressed most abundantly in the central nervous system (CNS) tissue and brain. The nature of the transmissible agent is not well understood. BSE possibly originated as a result of feeding of scrapie-containing sheep meat-and-bone meal to cattle.

In humans, the illness suspected of being foodborne is variant Creutzfeldt-Jakob disease (vCJD). The human vCJD and cattle BSE appear to be caused by the same agent. The neurodegenerative phase (build-up of $PrP^{res}$ isoform) of vCJD typically involves the formation of "daisy-shaped" areas of damage in the CNS, and there is also vacuolization (formation of holes) in the brain tissue that gives a spongy appearance when examined under a microscope. Cases of vCJD present with psychiatric problems, such as depression. As the disease progresses, neurological signs appears including unpleasant sensations in the limbs/face, problems with walking and muscle coordination, forgetfulness, among others. Late in the course of the disease patients are hospitalized until death.

The most reliable means for diagnosis of the human disease vCJD is the microscopic examination (a post-mortem procedure). Preliminary diagnosis of vCJD is based on patient history, clinical symptoms, electroencephalograms, and magnetic resonance imaging of the brain. This disease is rapidly progressive and fatal. CDC has used several mechanisms to conduct surveillance for vCJD and, during 1996-97, established the National Prion Disease Pathology Surveillance Center (NPDPSC) at Case Western Reserve University, Cleveland, Ohio. NPDPSC provides advanced neuropathologic and biochemical diagnostic services free of charge to physicians and state and local health departments.

The methods of diagnosis in cattle include immunohistochemistry (using antibody/antigen staining of post-mortem biopsy tissue), SAF-Immunoblot of brain, and Western Blots techniques, to name a few.

The major concern for consumer is the potential contamination of meat product by BSE contaminated tissues or the inclusion of BSE contaminated tissues in foods, including dietary supplements. High risk tissues for BSE contamination include the cattle's skull, brain, spinal cord, dorsal root ganglia, and the distal ileum of the small intestine. The direct or indirect intake of high-risk tissues may have been the source of human illness.

The U.S. experience of the BSE confirmed positive from a dairy cow in Washington State (December 2003) triggered a series of actions by the Secretary of Agriculture. In response to this event, in January 2004, FSIS issued three interim regulations and a notice in the Federal Register. The purpose of these policy issuances is to minimize human exposure to the BSE agent. For more information on this FSIS policies, resent issuances and surveillance program refer to the module titled "Bovine Spongiform Encephalopathy (BSE): Key Points for the Public Health Veterinarian" in the PHV Training materials.

**Emerging Foodborne Pathogens of Concern from the Food Industry Perspective**

***Campylobacter jejuni***

*Campylobacter jejuni* is a Gram-negative slender, curved, and motile rod. It is a microaerophilic organism, which means it has a requirement for reduced levels of oxygen and requires 3 to 5% oxygen and 2 to 10% carbon dioxide for optimal growth. The isolation of this pathogen requires special antibiotic-containing media and a special microaerophilic atmosphere (5% oxygen). *Campylobacter jejuni* is relatively fragile and sensitive to environmental stresses such as 21% oxygen, drying, heating, disinfectants,

and acidic conditions. This microorganism can grow at temperatures between 25-42˚C (77-107˚F), pH range of 5.5-8, and $a_w$ >0.95.

This bacterium is now recognized as an important pathogen. The pathogenic mechanisms of *C. jejuni* are still not completely understood, however, research has demonstrated that a series of virulence factors come into play for the pathogen to be able to cause disease. These factors include motility, chemotaxis, invasins, and adhesins, among others. Some investigators have shown that *C. jejuni* firstly colonizes the jejunum and ileum, and then the colon producing a heat-labile toxin (*Campylobacter* invasion antigens or Cia proteins) that may cause diarrhea.

Campylobacteriosis is the name of the illness caused by the pathogen *C. jejuni* and it is also often known as campylobacter enteritis or gastroenteritis. It is one of the most common bacterial causes of diarrheal illness (even more than *Shigella* spp and *Salmonella* spp combined) in the United States. Active surveillance through FoodNet indicates about 15 cases per 100,000 persons are diagnosed each year. Many more cases go undiagnosed or unreported, and it is estimated that this illness affect over 1 million persons every year and it is estimated that approximately 100 persons with *Campylobacter* infections may die. Campylobacteriosis occurs more frequent in the summer months than in the winter.

Although anyone can become ill with campylobacteriosis, children under 5 years and young adults (15-29) are more frequently afflicted than other age groups. *Campylobacter jejuni* infection causes diarrhea, which may be watery or sticky and can contain blood (usually occult) and fecal leukocytes. Other symptoms often present are fever, nausea, cramping, abdominal pain, headache, and muscle pain within 2-5 days after exposure to the organism. A very small number of the pathogen (fewer than 500) can cause illness in humans. The illness generally lasts 7-10 days and individuals with compromised immune systems the pathogen occasionally spreads to the bloodstream and causes a serious life-threatening infection.

Since *C. jejuni* is an invasive organism long-term effects of this illness can lead to Guillain-Barré syndrome, a rare disease that affects the nerves of the body beginning several weeks after the diarrheal illness. This disease occurs when a person's immune system is triggered to attack the body's own nerves, and can lead to paralysis that last several weeks and usually require intensive care. It is estimated that approximately one in every 1000 reported Campylobacteriosis cases leads to Guillain-Barré syndrome (40% of the syndrome cases).

Many chicken flocks are silently infected with *Campylobacter*, i.e., the chickens are infected with the organism but show no sign of infection and can be easily spread from bird to bird through a common water source or contact with infected feces. When infected chickens are slaughtered, the organism can be transferred from the intestines to the meat. More than half of the raw chicken in the United States market has *Campylobacter* on it. *Campylobacter* is also present in the giblets, especially the liver.

Raw milk, raw beef and pork are also sources of infection. The bacteria are often carried by healthy cattle, birds, and by flies on farms. Non-chlorinated water may also be a source of infections.

In 1982, CDC began a national surveillance program and a more detailed active surveillance was instituted in 1996; this will provide more information on how often the disease occurs and what risk factors are for getting it. The U.S. Department of Agriculture is conducting research on how to prevent the infection in chickens. Moreover, during the year 2006 FSIS started a nationwide young chicken and turkey microbiological baseline data collection program to acquire information concerning the prevalence and quantitative levels of selected foodborne pathogens including *Campylobacter.*

***Staphylococcus aureus***

*Staphylococcus aureus* is a Gram-positive bacterium (coccus) which on microscopic examination appears in clusters resembling grapes. It is a non-motile, non-spore forming facultative anaerobe that grows by aerobic respiration or by fermentation yielding lactic acid. This microorganism can grow at a temperatures between 7-45˚C (35.9-113˚F; optimum 37˚C [98.3˚F), pH range of 4.2-9.3 (depend on the type of acid present), at NaCl concentrations as high as 25%, and it is resistant to drying capable of producing enterotoxins in foods with $a_w$ as low as 0.85.

*Staphylococcus aureus* should be considered a potential pathogen and its pathogenesis is multifactorial since it can expresses many potential virulence factors like surface proteins (laminin, fibronectin, clumping factor, and an adhesion) that promote colonization in host tissue; invasins (leukocidin, kinases, and hyaluronidase) that promote bacterial spread in tissues; surface factors (capsule and Protein A) which inhibit phagocytic engulfment; carotenoids and catalase production that enhance their survivor; and membrane damaging toxins ($\alpha$-hemolysin, leukotoxin, leukocidin) that lyses eukaryotic cell membranes. There are other virulence factors including the exotoxins (enterotoxins of antigenic type SE A-G, Toxic Shock Syndrome Toxin-1, and Exfoliating Toxin) that damage host tissues or provoke symptoms of disease. The heat-stable enterotoxin A (SE A) is the most toxic and is responsible for causing diarrhea and vomiting when ingested and for staphylococcal food poisoning (staphyloenterotoxicosis or staphyloenterotoxemia).

One of the biggest concerns of this pathogen is the increase incidence of Methicillin resistant *S. aureus* (MRSA) and other strains that are resistant to a variety of different antibiotics. Furthermore, *S. aureus* strains can exhibit resistance, as a survival mechanism in the hospital environment, to antiseptic and disinfectants including quaternary ammonium compounds.

All people are believed to be susceptible to this type of bacterial intoxication; however, intensity of symptoms may vary. Death from staphylococcal food poisoning is very rare, although such cases have occurred among the elderly, infants, and severely debilitated persons. The onset of symptoms in staphylococcal food poisoning is usually rapid (30 min-8 hrs) and in many cases acute, depending on individual susceptibility to the toxin, the amount of contaminated food eaten, the amount of toxin in the food ingested, and the general health of the victim. The most common symptoms are nausea, vomiting, retching, abdominal cramping, and prostration. Some individuals may not always demonstrate all the symptoms associated with the illness. In more severe cases, headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur. An enterotoxin A dose of less than 1.0 microgram in contaminated food will

produce symptoms of staphylococcal intoxication and this toxin level is reached when *S. aureus* populations exceed 100,000 cells per gram. Recovery generally takes two days however it is not unusual for complete recovery to take three days and sometimes longer in severe cases.

The true incidence of staphylococcal food poisoning 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* toxin); inadequate collection of samples for laboratory analyses; and improper laboratory examination. Thus, in the diagnosis of staphylococcal foodborne illness, proper interviews with the victims and gathering and analyzing epidemiologic data are essential. Incriminated foods should be collected and examined for staphylococci. The presence of relatively large numbers of enterotoxigenic staphylococci is good circumstantial evidence that the food contains toxin. The most conclusive test is the linking of an illness with a specific food or in cases where multiple vehicles exist, the detection of the toxin in the food sample(s).

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

Staphylococci exist in air, dust, sewage, water, milk, and food or on food equipment, environmental surfaces, humans, and animals. Humans and animals are the primary reservoirs. Animals and poultry carry *S. aureus* on parts of their body which can lead to infections. Cow's udder and teats, tonsils and skin of pigs, and skin of chickens and turkeys are known sources. Staphylococci are present in the nasal passages and throats and on the hair and skin of 50 percent or more of healthy individuals. This incidence is even higher for those who associate with or who come in contact with sick individuals and hospital environments. Although food handlers are usually the main source of food contamination in food poisoning outbreaks, equipment and environmental surfaces can also be sources of contamination with *S. aureus*.

*Staphylococcus aureus* is highly vulnerable to destruction by heat treatment and nearly all sanitizing agents when correctly applied. While heat processing (pasteurization) and normal cooking temperatures are effective to kill the pathogen, food establishments have to be alert that the enterotoxins are heat-stable (extremely resistant to heat) and are not inactivated by heat. There are guidelines for the industry to ensure that the processing steps they are using are adequate to meet their particular food safety objectives. Heat resistance is increased in dry, high-fat and high-salt foods, and survives frozen storage. Thus, the presence of this bacterium or its enterotoxins in processed foods or on food processing equipment (in areas that are difficult to clean) is generally an indication of poor sanitation and processing practices. Foods which present the greatest risk are those in which a heat treatment has been applied (e.g. cooking) or application of an inhibitory agent or treatment (e.g. cured, salted meats). Foods are examined for the presence of *S. aureus* and/or its enterotoxins to confirm that *S. aureus* is the causative agent of foodborne illness, to determine whether a food is a potential source of

staphylococcal food poisoning, and to demonstrate post-processing contamination, which is generally due to human contact or contaminated food-contact surfaces. The presence of a large number of *S*. *aureus* organisms in a food may indicate poor handling or sanitation; however, it is not sufficient evidence to incriminate a food as the cause of food poisoning. The isolated *S*. *aureus* must be shown to produce enterotoxins.

As mentioned previously, this pathogen can cause severe food poisoning and it has been identified as the causative agent in many outbreaks by eating foods in which enterotoxin has been produced because of time and temperature abuse, poor sanitation during processing, or other factors. *Staphylococcus aureus* is probably responsible for even more cases in individuals and family groups than the records show. Recently, this pathogen has been implicated in two Class I (high health risk) recalls. The first one took place in Washington State (November 22, 2005) where the firm recalled various sized vacuum-packed packages of fully cooked honey cured ham and smoked beef strips (approximately 340 pounds overall) that may had contained *S*. *aureus* enterotoxin. The problem was discovered by FSIS and no illnesses were reported. The second recall took place in New Jersey on June 13, 2006 where the importing firm voluntarily recalled approximately 664 pounds of boneless Prosciutto ham that also may had contained *S*. *aureus* enterotoxin. The problem was discovered through testing done by the Canadian Food Inspection Agency. FSIS did not receive any reports of illness associated with the consumption of this product.

In a resent study done in the Netherlands, a new MRSA clone related to swine and cattle farming were detected and this clone was also isolated in meat products. Contamination of food products can be traced back to slaughter plants and to poor sanitary conditions. This is another example of a major factor of contamination resulting in transfer of pathogenic agents between countries (import/export).

**Norwalk and Norwalk-like viruses**

Viruses are inert particles that can pass from host to host. Since these particles are completely inert, they cannot multiply in foods or outside the host, cannot carry out any metabolic activity, nor respond to stresses encountered in the environment. Nevertheless, viruses have emerged as causes of foodborne disease.

Norwalk virus is the prototype strain of genetically and antigenically diverse single stranded ribonucleic acid (RNA) viruses which is classified in the genus Norwalk-like in the family *Caliciviridae.* The family consist of several serological distinct groups of viruses that have been named after the places where the outbreaks occurred (i.e., in the U.S. the Norwalk virus was the first gastroenteritis virus in Norwalk County). Norwalk-like viruses (NLVs) have the ability to survive in relatively high levels of chlorine and varying temperatures (i.e., from freezing to 60˚C [140˚F]).

Common names of the illness caused by Norwalk and NLVs are viral gastroenteritis, acute nonbacterial gastroenteritis, food poisoning, and food infection. The virus has an incubation period of 12-48 hrs after consumption of contaminated food or water and lasts for 1-2½ days. The illness is self-limiting, mild, and characterized by acute onset of nausea, vomiting, abdominal cramps, and diarrhea. Vomiting is more prevalent in children whereas diarrhea is common to adults. The infectious dose is unknown but presumed to be low (less than 100 viral particles).

Theoretically, any food item can potentially be infected with NVL through fecal contamination; certain foods are implicated more than others in outbreaks of NLV gastroenteritis, like shellfish. Also, food contamination by infectious food handlers is another frequent cause of outbreaks (RTE foods like salads and deli sandwiches). Other vehicles of transmission include water (from municipal supplies, wells, etc.) and person-to-person spread (nursing homes and day care centers).

Because of the antigenic and genetic diversity of NLVs various diagnostic methods have been developed to identify NLVs in clinical specimens. The most common ones include electron microscopy, immune electron microscopy, enzyme Immunoassays (ELISA), nucleic acid hybridization, and reverse transcription-polymerase chain reaction; the last two methods are assays to detect NLV genome in clinical and environmental specimens.

## FOODBORNE DISEASE OUTBREAKS

An outbreak of foodborne illness occurs when a group of people consume the same contaminated food and two or more of the individuals develop the same symptoms or illness. For example, it may be a group that ate a meal together somewhere, or it may be a group of people who do not know each other at all, but who all happened to buy and eat the same contaminated item from a grocery store or restaurant.

For an outbreak to occur, an event or combination of events must happen to contaminate a batch of food eaten by a group of people. For example, contaminated food may be left out at room temperature for many hours, allowing the bacteria to multiply to high numbers, and then not properly cooked to kill the bacteria.

Many outbreaks are local in nature. For examples, a catered meal at a reception, a pot-luck supper, or eating a meal at an understaffed restaurant on a particularly busy day. These outbreaks are recognized when a group of people realize that they all became ill after a common meal, and someone calls the local health department.

However, outbreaks are increasingly being recognized that are more widespread, that affect persons in many different places, and that are spread out over several weeks. As an illustration, in 2002, a salmonellosis outbreak was traced to persons who consumed ground beef in five states. Forty seven cases were identified where 17 people were hospitalized and one died. The outbreak was recognized because it was caused by a multidrug-resistant *Salmonella* Newport and fingerprinting pattern of 94% of the isolates were indistinguishable indicating that the outbreak was originated by the same bacterial strain.

The vast majority of reported cases of foodborne illnesses is not part of recognized outbreaks, but occurs as individual or "sporadic" cases. It may be that many of these cases are actually part of unrecognized widespread or diffuse outbreaks.

The initial clue that an outbreak is occurring can come in various ways:

- It may be when a person realizes that several other people who were all together at an event have become ill and he or she calls the local health department.
- It may be when a physician realizes she has seen more than the usual number of patients with the same illness.

- It may be when a county health department gets an unusually large number of reports of illness.

Once an outbreak is detected, an investigation begins. The outbreak is systematically described by time, place, and person by interviewing people, gathering epidemiological information, testing implicated food vehicle, and other associated information. If the causative microbe is not known, samples of stool or blood are collected from ill people and sent to the public health laboratory to make the diagnosis.

Detecting and investigating such widespread outbreaks is a major challenge to our public health system. This is the reason that new and more sophisticated laboratory methods are being developed and used by CDC and in state public health department laboratories.

## EPIDEMIOLOGY

One of the public health strategies for dealing with foodborne illness outbreaks is the use of epidemiology. Epidemiology is the study of factors determining and influencing the frequencies and distribution of a disease, injury, and other health-related events and their causes in a defined human population. The purpose is to establish programs to prevent and control their development and spread. Let's review a few very basic principles.

- The term "epidemic" is used when there is an occurrence of more cases of disease than expected in a given area or among a specific group of people over a particular period of time.
- The term "endemic" refers to the usual prevalence of a given disease or agent in a population or geographic area at all times.

FSIS employs a group of epidemiologists to assist in investigating foodborne disease outbreaks related to meat, poultry, and egg products.

### Surveillance systems for tracking foodborne diseases

The hardest outbreaks to detect are those that are spread over a large geographic area, with only a few cases in each state. These outbreaks can be detected by combining surveillance reports at the regional or national level and looking for increases in infections of a specific type.

CDC is part of the U. S. Public Health Service, with a mission to use the best scientific information to monitor, investigate, control and prevent public health problems. CDC works closely with state health departments to monitor the frequency of specific diseases and conducts national surveillance for them. CDC provides expert epidemiologic and microbiologic consultation to health departments and other federal agencies on a variety of public health issues, including foodborne disease. CDC can also send a team into the field to conduct emergency field investigations of large or unusual outbreaks, in collaboration with state public health officials.

CDC researchers develop new methods for identifying, characterizing and fingerprinting the microbes that cause disease. It translates laboratory research into practical field methods that can be used by public health authorities in States and counties.

CDC is not a regulatory agency. Government regulation of food safety is carried out by the Food and Drug Administration, the Food Safety and Inspection Service (USDA), the National Marine Fisheries Service, and other regulatory agencies. CDC maintains regular contact with the regulatory agencies. Although it does not regulate the safety of food, the CDC assesses the effectiveness of current prevention efforts. It provides independent scientific assessment of what the problems are, how they can be controlled, and of where there are gaps in our knowledge.

*FoodNet (Foodborne Disease Active Surveillance Network)*

FoodNet consists of active surveillance for foodborne diseases and related epidemiologic studies designed to help public health officials better understand the epidemiology of foodborne diseases in the United States. It is the principal foodborne disease component of CDC's Emerging Infections Program (EIP). It is a collaborative project of the CDC, ten EIP sites (California, Colorado, Connecticut, Georgia, New York, Maryland, Minnesota, Oregon, Tennessee and New Mexico), the USDA, and the Food and Drug Administration.

FoodNet provides a network for responding to new and emerging foodborne diseases of national importance, monitoring the burden of foodborne diseases, and identifying the sources of specific foodborne diseases.

The FoodNet methods by this surveillance network consist of establishing laboratory-confirmed cases of infection from each site. A case report is completed which includes information on demographics, clinical outcomes, and the pathogen.

*PulseNet (The Molecular Subtyping Network for Foodborne Bacterial Disease Surveillance)*

PulseNet is the national molecular subtyping network for foodborne disease surveillance and allows state laboratories and CDC to compare strains of pathogenic bacteria from all across the United States to detect widespread outbreaks. It is the CDC’s network of public health laboratories that perform a DNA "fingerprinting" method called pulsed-field gel electrophoresis (PFGE) on foodborne bacteria. PulseNet is a national network of public health laboratories that provides an early warning system for outbreaks of foodborne disease.

PFGE is a molecular method where bacterial chromosomal DNA is digested with specific restriction enzymes (at least two); the digested fragments are then inserted into an agarose gel and separated in an electrical field (electrophoresis). The electrophoretic patterns are visualized following staining with a specific dye and the image is captured using commercially available digital systems. The data analysis can be performed by using software programs, and the PFGE typing criteria employed to determine the genetic relatedness among strains of particular bacterial specie is correlated with the similarities in the DNA banding pattern.

The network identifies and labels each "fingerprint" pattern and permits rapid comparison of these patterns through an electronic database at the CDC to identify related strains. At present, PulseNet tracks four foodborne disease-causing bacteria: *E. coli* O157:H7, nontyphoidal *Salmonella*, *Shigella*, and *Listeria monocytogenes* at the DNA level.

The spectrum of foodborne diseases is constantly changing. A century ago, typhoid fever, tuberculosis and cholera were common foodborne diseases. Improvements in food safety, such as pasteurization of milk, safe canning, and disinfection of water supplies have conquered those diseases.

Newly recognized microbes emerge as public health problems for several reasons: microbes can easily spread around the world, new microbes can evolve, the environment and ecology are changing, food production practices and consumption habits change, and because better laboratory tests can now identify microbes that were previously unrecognized.

In the last 15 years, several important diseases of unknown cause have turned out to be complications of foodborne infections. For example, we now know that the Guillain-Barré syndrome can be caused by *Campylobacter* infection, and that the most common cause of acute kidney failure in children, hemolytic uremic syndrome, is caused by infection with *E. coli* O157:H7 and related EHEC pathogens. In the future, other diseases whose origins are currently unknown may turn out be related to foodborne infections.

## FOOD PROCESSING ESSENTIALS

This section isn't intended to cover each type of food preservation method in detail. It is intended to remind you of the types of food preservation that are currently practiced and to point out methods of preservation that you may be exposed to as the IIC in a facility. You will remember some of this from the section of your training on the Regulated Industries. Proper processing of food helps to ensure that the growth of harmful microorganisms is controlled, reduced, or eliminated.

### Preservation of foods

The basic principle of all forms of food preservation is to either slow down the activity of disease causing bacteria, or to kill the bacteria altogether so that they do not cause illness in the consumer of the product.

Following is a list of food preservation techniques commonly used. Not all of these are present in a FSIS inspected slaughter/processing facility. But as a PHV you will most likely be exposed to the use of refrigeration, freezing, heat, and chemical preservation in the slaughter house and processing environment, at a minimum. Irradiation is just starting to be more accepted by the public, there are a few irradiation facilities in the United States.

Types of food preservation:

- Refrigeration and freezing
- Canning

- Irradiation
- Chemical preservation
- Pasteurizing (heat)
- Pickling
- Salting
- Dehydration
- Fermentation
- Carbonation
- Cheese-making
- Freeze-drying

When you first report to your duty station it will be important to review the HACCP and SSOP plans and take a tour of the facility to familiarize yourself with the processes of the establishment. If you have questions about the processes, you can ask the establishment, or you may contact the Technical Service Center for technical guidance.

## OVERVIEW OF FSIS MICROBIOLOGICAL TESTING PROGRAMS

This section will provide a brief overview of FSIS microbiological testing programs. You will either perform or be involved with these testing programs in the establishment. This is a very brief overview. You covered all of these in detail when you attended the FSRE training. Remember that the establishment may have its own microbiological testing program. You are to regularly review the records associated with the plant's testing program when the testing program has relevance to the plant's food safety systems.

FSIS conducts microbiological testing for *Salmonella, E. coli* O157:H7, and *Listeria monocytogenes*. FSIS also has performance standards for *Salmonella*, and a pathogen reduction regulation that requires some plants to conduct *E. coli* generic testing.

*Salmonella* Performance Standards

First, let's review the performance standards for *Salmonella*. The requirements for livestock and poultry establishments are covered in 9 CFR 310.25(b) and 381.94(b), respectively. FSIS Directive 10,011.1 Attachment 1 contains instructions for the Salmonella performance standards. Attachment 1 also provides background information and answers to questions regarding FSIS Directive 10,011.1. FSIS Directive 10,230.5 is the self-Instruction guide for collecting raw meat and poultry product samples for Salmonella analysis for establishments.

Generic *E. coli* testing by establishments

The pathogen reduction regulation also covered a requirement for some plants to conduct generic *E. coli* testing and is done by the establishment [9 CFR 310.25(a) and 381.94 (a)]. Generic *E. coli* is an indicator organism that gives an indication if the establishment's sanitary dressing procedures are working effectively. The samples can be collected using several methods (sponge, whole bird rinse, excision) depending on the type of carcass. FSIS has provided resources to assist plants including "Guidelines of *Escherichia coli* Testing for Process Control Verification in Cattle and Swine Slaughter Establishments," and "Guidelines of *Escherichia coli* Testing for Process Control

Verification in Poultry Slaughter Establishments". These resources can be found in the FSIS Website.

*E. coli* O157:H7

*Escherichia coli* O157:H7 is one of the pathogens that are included in the FSIS testing program. While *E. coli* infections do not cause the largest numbers of illnesses, the illness due to this pathogen is very severe and death can result. The CDC now estimates that foodborne transmission of the pathogen annually causes 73,000 illnesses, resulting in more than 2,000 hospitalizations and 60 deaths. This represents an economic burden where the annual cost (2003) of illness due to this pathogen was in the vicinity of $405 million dollars. FSIS issued a final rule requiring establishments to conduct a reassessment of their HACCP plans for E. coli O157:H7. Here are some recent agency policy issuances on *E. coli* O157:H7 and can be access through the Web: "http://www.fsis.usda.gov/Regulations_&_Policies/Index.asp".

- FSIS Directive 10,010.1, Revision 1 – Microbiological Testing Program for Escherichia coli O157:H7 in Raw Ground Beef Products and Raw Ground Beef Components and Beef Patty Components

- Federal Register Docket No. 04-042N (May 26, 2005/Rules and Regulations) – HACCP Plan Reassessment for Mechanically Tenderized Beef Products.

- FSIS Notice 17-07 – Follow-up Sampling of Certain Raw Ground Beef Products after an FSIS Verification Sample Tests Positive for *E. coli* O157:H7.

- FSIS Notice 18-07 – Routine Sampling of Beef Manufacturing Trimmings Intended for Use in Raw Ground Beef.

- FSIS Notice 62-07 – Instructions for Verification Sampling Programs for *E. coli* O157:H7 in Raw Beef Products.

- FSIS Notice 65-07 – Notice of Reassessment for *E. coli* O157:H7 Control and Completion of a Checklist for all Beef Operations.

- FSIS Notice 66-07 – Multiple Follow-up Sampling After FSIS Positive *E. coli* O157:H7 Results.

- FSIS Notice 68-07 – Routine Sampling and Testing of Raw Ground Beef Components Other than /trim and Imported Raw Ground Beef Components for *E. coli* O157:H7.

*Listeria monocytogenes*

During the 1980's, *L. monocytogenes* began to emerge as a problem in processed meat and poultry products. In the 1990's there were outbreaks of foodborne illness in which hotdogs, and possibly deli (luncheon) meats, were implicated.

Since 1999-2003, FSIS published Federal Register Notices and FSIS Notices, held public meetings, and developed *Listeria* Guidelines for the industry. The FSIS risk

assessment, in conjunction with a previously released FDA/FSIS risk ranking and public comment gathered on the topic, provided important data enabling FSIS to design a final *L. monocytogenes* rule. This rule was published on June 6, 2003 and became effective on October 6, 2003.

9 CFR 430 states that Lm can contaminate RTE products that are exposed to the environment after a lethality treatment (destroy/kill). Lm is a hazard that an establishment must control through its HACCP plan, or prevent in the environment through a SSOP or other prerequisite program if it produces RTE product that is exposed post-lethality. RTE product is adulterated if it contains Lm or if it contacts surfaces contaminated with *Lm*. In order to maintain sanitary conditions necessary to meet this requirement, an establishment producing post-lethality exposed RTE product must comply with one of three alternatives outlined in the regulation.

Following is a very brief summary of how establishments must meet the requirements of regulation 430. If an establishment chooses Alternative 1 they must use a post-lethality treatment that reduces or eliminates microorganisms on product and an antimicrobial agent or process that suppresses or limits the growth of *Lm*. If an establishment chooses alternative 2 they can use either the post-lethality treatment of product or antimicrobial agent or process that suppresses or limits growth; however if an establishment chooses the antimicrobial agent or process they must also have a sanitation program that addresses the testing of food contact surfaces. For Alternative 3, it employ sanitation measurements only and there is a higher potential risk of post lethality contamination of the product with *Lm*, therefore FSIS will most likely sample at a higher frequency than for Alternative 2 or 1. The risk of contamination with Alternative 2 is higher than the risk of contamination with Alternative 1. Theoretically, Alternative 1 should produce the safest product, and therefore, this product will be subject to the lowest frequency of verification testing by FSIS.

The final rule contains a great deal of background information on *Listeria* contamination of RTE product. The Directive contains instructions for inspectors who will be verifying compliance with the new 430 regulation, as well as procedures for collecting product samples. The Directive that is accessible through the FSIS website contains five attachments and four resources on *Listeria*. Together there is probably several hundred pages of information regarding how the new regulation will be implemented, who it applies to, and information companies should consider when addressing Lm in their food safety systems. FSIS also put out a CD-ROM for inspectors with this same material on it as a reference for field inspectors (FSIS Directive 10,240.4: Resources and Compliance Guidelines for Controlling *Lm*, October 2003, Center for Learning).

Agency policies are updated as information develops about the prevalence of pathogens, foodborne illness outbreaks, and industry practices. It is your responsibility to maintain a current knowledge of Agency policies and how they affect your job duties. This basic understanding of food microbiological principles will also help you as you perform your regulatory responsibilities.

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