



Bacteria Associated with Foodborne Diseases

This IFT Scientific Status Summary discusses *Salmonella*, *Shigella*, *Campylobacter*, *Listeria*, and *Vibrio* species, and *Yersinia enterocolitica*, *Staphylococcus aureus*, *Clostridium perfringens*, *Clostridium botulinum*, *Bacillus cereus*, and *Enterobacter sakazakii*.

This Scientific Status Summary is an updated review of the bacteria of primary significance in foodborne disease, their significance as pathogens, their association with foods, and related control measures. The original publication appeared in the April 1988 edition of *Food Technology*.

An estimated 76 million cases of foodborne illness occur each year in the United States, costing between \$6.5 and \$34.9 billion in medical care and lost productivity (Buzby and Roberts, 1997; Mead et al., 1999). In the United States, incidence of foodborne illness is documented through FoodNet, a reporting system used by public health agencies that captures foodborne illness in over 13% of the population. Of the 10 pathogens tracked by FoodNet, *Salmonella*, *Campylobacter*, and *Shigella* are responsible for most cases of foodborne illness. When both the estimated number of cases and mortality rate are considered for bacterial, viral, and parasitic cases of foodborne illness, *Salmonella* causes 31% of food related deaths, followed by *Listeria* (28%), *Campylobacter* (5%), and *Escherichia coli* O157:H7 (3%) (Mead et al., 1999).

Data are only available for confirmed cases, and it is generally accepted in the scientific community that the true incidence of foodborne disease is underreported. Of the estimated 13.8 million cases of foodborne illness due to known agents, roughly 30% are due to bacteria. The remaining cases of known etiology are due to parasites in 3% of the cases and viruses in 67% of the cases (Mead et al., 1999). Because parasites (Orlandi et al., 2002), *E. coli* O157:H7 (Buchanan and Doyle, 1997), and viruses (Cliver, 1997) have been addressed by previously published Scientific Status Summaries, these pathogens are not discussed here. *Enterobacter sakazakii*, an emerging pathogen of concern in infant formula, is a new inclusion in this revised Scientific Status Summary.

Scientific Status Summary

Bacteria are the causative agents of foodborne illness in 60% of cases requiring hospitalization (Mead et al., 1999). The international impact of foodborne illness is difficult to estimate. However, about 2.1 million children in developing countries die due to diarrheal-related illnesses annually. It is suspected that food or water is the vehicle for many of these illnesses (WHO, 2002).

Because food is biological in nature and is capable of supplying consumers with nutrients, it is equally capable of supporting the growth of contaminating

microorganisms. Three types of bacterial foodborne diseases are recognized: intoxications, infections, and toxicoinfections. Foodborne bacterial intoxication is caused by the ingestion of food containing preformed bacterial toxin, such as the toxins produced by *Staphylococcus aureus* and *Clostridium botulinum*, resulting from bacterial growth in the food. Foodborne infection, on the other hand, is caused by ingestion of food containing viable bacteria such as *Salmonella* or *Listeria* which then grow and establish themselves in the host, re-

sulting in illness. Foodborne toxicoinfections result when bacteria present in food, such as *Clostridium perfringens*, are ingested and subsequently produce a toxin in the host.

Some pathogens reside in the intestinal tracts of normal, healthy animals and, in some instances, humans. Certain microorganisms are ubiquitous in nature, occurring on soil and vegetation, in animal wastes, and on animal carcasses. Human skin surfaces and nasal passages harbor staphylococci. Water supplies may contain pathogens when con-

Table 1— Foodborne Disease in the United States, Including Estimated Annual Prevalence. Adapted from IFT (2000).

Bacteria	Principal symptoms ^a	Potential food contamination ^a	No. of illnesses ^b	No. of deaths ^b
<i>Bacillus cereus</i>	Diarrheal—watery diarrhea, abdominal cramps and pain Emetic—nausea and vomiting	Meats, milk, vegetables, fish Rice products, starchy foods	27,360 n/a	0 n/a
<i>Campylobacter</i> spp.	Watery diarrhea, fever, abdominal pain, nausea, headache, muscle pain	Raw chicken, beef, pork, shellfish, raw milk	1,963,141	99
<i>Clostridium botulinum</i>	Weariness, weakness, vertigo, double vision, difficulty swallowing and speaking	Improperly canned or fermented goods	58	4
<i>Clostridium perfringens</i>	Intense abdominal cramps, diarrhea	Meat, meat products, gravies	248,520	7
<i>Enterobacter sakazakii</i> ^c	Meningitis, enteritis	Powdered infant formula	1:100,000 infants ^c	20-50% ^c
<i>Listeria monocytogenes</i>	Nausea, vomiting, diarrhea; influenza-like symptoms, meningitis, encephalitis; septicemia in pregnant women, their fetuses, or newborns; intrauterine or cervical infection that may result in spontaneous abortion or stillbirth	Raw milk, cheeses (particularly soft ripened varieties), raw vegetables, raw meats, raw and smoked fish, fermented sausages	2,493	499
<i>Salmonella</i> Typhi and <i>Salmonella</i> Paratyphi	Typhoid-like fever, malaise, headache, abdominal pain, body aches, diarrhea, or constipation	Raw meats, poultry, eggs, milk and dairy products, fish, shrimp, yeast, coconut, sauces, salad dressings (i.e., homemade items containing unpasteurized eggs and no or insufficient acidification for destroying pathogens)	659	3
Other <i>Salmonella</i> spp.	Nausea, vomiting, abdominal cramps, fever, headache; chronic symptoms (e.g., arthritis)	Raw meats, poultry, eggs, milk and dairy products, fish, shrimp, yeast, coconut, sauces, salad dressings (i.e., homemade items containing unpasteurized eggs and no or insufficient acidification for destroying pathogens)	1,341,873	553
<i>Shigella</i> spp.	Abdominal pain and cramps; diarrhea; fever; vomiting; blood, pus, or mucus in stools, tenesmus	Salads (potato, tuna, chicken, macaroni) raw vegetables, bakery products (e.g., cream-filled pastries), sandwich fillings, milk and dairy products, poultry	89,648	14
<i>Staphylococcus aureus</i>	Nausea, vomiting, retching, abdominal cramps, prostration	Meat and meat products, poultry, egg products, salads (chicken, potato, macaroni), cream-filled bakery products, milk and dairy products	185,060	2
<i>Vibrio cholera</i> Serogroup 01	Mild watery diarrhea, acute diarrhea, rice-water stools	Raw or recontaminated oysters, clams, crabs	49	0
<i>V. cholera</i> Serogroup non-01	Diarrhea, abdominal cramps, fever, vomiting, nausea; blood or mucus-containing stools	Raw or recontaminated oysters, clams, crabs	49	0
<i>Vibrio vulnificus</i>	Fever, chills, nausea, septicemia in individuals with some underlying diseases or taking immunosuppressive drugs or steroids	Raw or recontaminated oysters, clams crabs	47	18
Other <i>Vibrio</i> spp.	Diarrhea, abdominal cramps, nausea, vomiting, headache, fever, chills	Raw, improperly cooked or recontaminated shellfish or fish	n/a	n/a
<i>Yersinia enterocolitica</i>	Fever, abdominal pain, diarrhea and/or vomiting	Meats, oysters, fish, raw milk	86,731	2

^aBased on FDA/CFSAN (2003b).

^bBased on Mead et al. (1999). n/a indicates that no estimate was provided for this pathogen.

^cFrom WHO (2004).

taminated with fecal matter. Coastal waters may also naturally harbor the pathogen *Vibrio vulnificus*. It is thus obvious how difficult it is to prevent one or more pathogens from contaminating foods.

The presence of potentially life-threatening pathogens in our environment, the ability of some of them to survive and/or proliferate under refrigeration and in reduced oxygen atmospheres, and, for some pathogens, the low number necessary for causing disease indicate the seriousness of the potential hazards with which we are faced. The food industry implements a variety of effective control measures to limit potential hazards. This generally begins on the farm with the implementation of good agricultural practices. Some raw products, such as poultry, are subject to performance standards in spite of the fact that the consumer will presumably cook the product before consumption. While all food manufacturers utilize control measures to ensure food safety, some food manufacturers are required to create and follow a Hazard Analysis and Critical Control Point (HACCP) plan. Critical control points identified through HACCP may include destruction or inactivation of the relevant bacteria or their spores through the use of heat treatments (e.g., pasteurization, canning), dehydration, freezing, refrigeration, specialized packaging, and/or approved antimicrobial preservatives. Additionally, extensive quality control procedures are maintained to ensure that these processes are effective.

It is impossible, however, to create a risk-free food supply. While food manufacturers and distributors employ necessary control measures to ensure the safety of food until it reaches the consumer, all food handlers and consumers have the responsibility upon purchase of the food to maintain these control measures until consumption. While outbreaks associated with a particular commercially processed food receive widespread public attention, a much greater number of individual cases of foodborne illness occurring in restaurants and in the home are not reported. The Centers for Disease Control and Prevention (CDC) reported that between 1993 and 1997, approximately 19% of foodborne illness outbreaks occurred in private residences. Numerous surveys have highlighted inadequate home food storage and handling prac-

tices as major contributors to foodborne illness (Altekruse et al., 1996; Meer and Misner, 2000). For most of the pathogens discussed in this Scientific Status Summary (Table 1), illness can be avoided by heating and cooling foods to the appropriate temperatures, storing foods at the appropriate conditions for the recommended period of time, hand-washing, and avoiding cross-contamination. Delicatessens, cafeterias, and restaurants were responsible for 33% of outbreaks (CDC, 2000b). Only one of more than 1,300 outbreaks, including bacterial and viral, with known or unknown etiology, was attributed to a "commercial product" in 2000 (CDC, 2000d). Proper handling, cooking, and storage practices in foodservice operations and in the home can prevent the majority of foodborne illnesses.

In the foodservice sector, education of the food preparer and server, with emphasis on good personal hygiene, is the best preventive measure. Unfortunately, the majority of foodservice workers are young (under 30 years of age), inexperienced, and stay on the job less than a year; thus, finding and educating these food handlers while they are actively working is difficult (Marth, 1985; National Restaurant Association, 2004).

However, training programs such as Servsafe®, conducted through the National Restaurant Association Education Foundation, and SuperSafeMark®, offered through the Food Marketing Institute, have a positive impact on retail food safety practices (Cotterchio et al., 1998; Lynch et al., 2003; Mathias et al., 1995).

Several studies and reviews have highlighted the contribution of changing demographics in the United States with the increased risk of foodborne illness (Knabel, 1995; Zink, 1997). The increase in the elderly population and individuals with weakened immune systems underscores the need for rigorous food safety efforts, both on the part of the food manufacturer and the consumer. Foodborne illnesses are more likely to be life-threatening for the immune compromised, the elderly, and individuals debilitated by underlying health problems such as cirrhosis, hepatitis, hemochromatosis, etc.

Consumer education and increased regulatory control of foodservice establishments through inspection and strict enforcement of proper food handling

practices probably have the greatest chances for success in controlling foodborne illness. The need for continual education of consumers and all food handlers concerning the significant hazards associated with foodborne pathogenic microorganisms and proper food handling procedures is evident. Regulatory control over food handling in the home is not possible, but increased consumer education could have a beneficial effect.

Salmonella

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Salmonella is a generic name applied to a group of nearly 2,000 biochemically related serotypes responsible for foodborne illness.

Significance as a Pathogen

The total number of cases of human salmonellosis have remained fairly constant between 1996 and 2002 (Figure 1), although the serotypes causing illness have changed (CDC, 2003a). Roughly two to four million cases of foodborne salmonellosis occur annually in the United States, and the estimated 1.3 million cases that occurred in 2000 cost \$2.4 billion in medical costs and lost productivity (USDA/ERS, 2003). Between 1988 and 1995 there were between 40,000 and 50,000 reported, confirmed cases of salmonellosis annually; since 1997, that number has been below 35,000 (CDC, 2004; FDA/CFSAN, 2003b).

The disease is grossly underreported because it is generally a self-limiting gastroenteritis which may be misdiagnosed as intestinal influenza by the patient or the physician. As a consequence, estimates of the true incidence of disease are based on assumptions derived from epidemiological evidence. Clearly, salmonellosis continues to be an important cause of foodborne disease worldwide.

Two clinical manifestations caused by *Salmonella* are recognized: enteric fever (a severe, life-threatening illness) and the more common foodborne illness syndrome. In both cases, the responsible microorganisms enter the body via the oral route.

Enteric fever, commonly referred to as typhoid fever, is primarily caused by one species, *Salmonella* Typhi, but other salmonellae such as *Salmonella* Paratyphi are potentially capable of pro-

Scientific Status Summary

ducing this syndrome. The illness is commonly associated with foreign travel and affects an estimated 800 people annually (Mead et al., 1999). Although the route of entry of the pathogen into the body is primarily oral, the symptoms of enteric fever are generally not elicited through the intestinal tract. However, a short episode of vomiting and diarrhea sometimes occurs in the first day or two in typhoid fever. The onset times vary considerably between typhoid and paratyphoid enteric fevers. Onset time for typhoid is usually 8–15 days, seldom as short as five days but sometimes as long as 30–35 days; while onset time for paratyphoid fever tends to be shorter, and may be so short as to suggest typical food poisoning (Parker, 1984). The usual symptoms of both are headache, malaise, anorexia, and congestion of the mucous membranes, especially of the upper respiratory tract. Bacteremia generally occurs in the first week of illness. In a study of 1,138 cases, Coleman and Buxton (1907) reported positive blood cultures in 89% of patients during the first week, 73% in the second week, 60% in the third, 38% in the fourth, and 26% after the fourth week. Arthritic symptoms may emerge three to four weeks after infection in roughly 2% of the cases. The 10% mortality rate of *Salmonella* Typhi and *Salmonella* Paratyphi is high compared to other *Salmonella* species (FDA/CFSAN, 2003b).

Typically, common foodborne illness

resulting from *Salmonella* infection is characterized by a self-limiting acute gastroenteritis. Contaminated food or water is the usual, but not the only, vehicle. The incubation period varies from six to 48 hr and generally falls within a range of 12–36 hr. Variation in the incubation time may be attributed to the size of the infecting dose, the virulence (degree of pathogenicity) of the microorganisms, the susceptibility of the host, and the physicochemical composition of the transmitting food. As few as 15 cells can cause illness (FDA/CFSAN, 2003b). Symptoms include diarrhea, abdominal cramps, vomiting, and fever, which generally last from one to seven days. However, the microorganisms may be excreted in the feces for many weeks after symptoms subside. Although the illness is usually self limiting, there is a 15% mortality rate in elderly who have developed septicemia due to *Salmonella dublin*, and a 3.6% mortality rate in nursing home cases of *Salmonella* Enteritidis (FDA/CFSAN, 2003b). Salmonellosis may be confused clinically with staphylococcal intoxication, but there are important distinctions. *Salmonella* has a longer incubation period than staphylococci (usually 12–36 hr vs. 2–4 hr) and is usually accompanied by fever, which is absent in staphylococcal intoxication. And unlike *Salmonella* food poisoning, the acute symptoms of staphylococcal food poisoning normally disappear within 24 hours.

In some cases, gastroenteritis may be followed by extraintestinal invasion resulting in enteric fever, which is more likely to occur in the very young, the aged, and debilitated patients.

Association with Foods

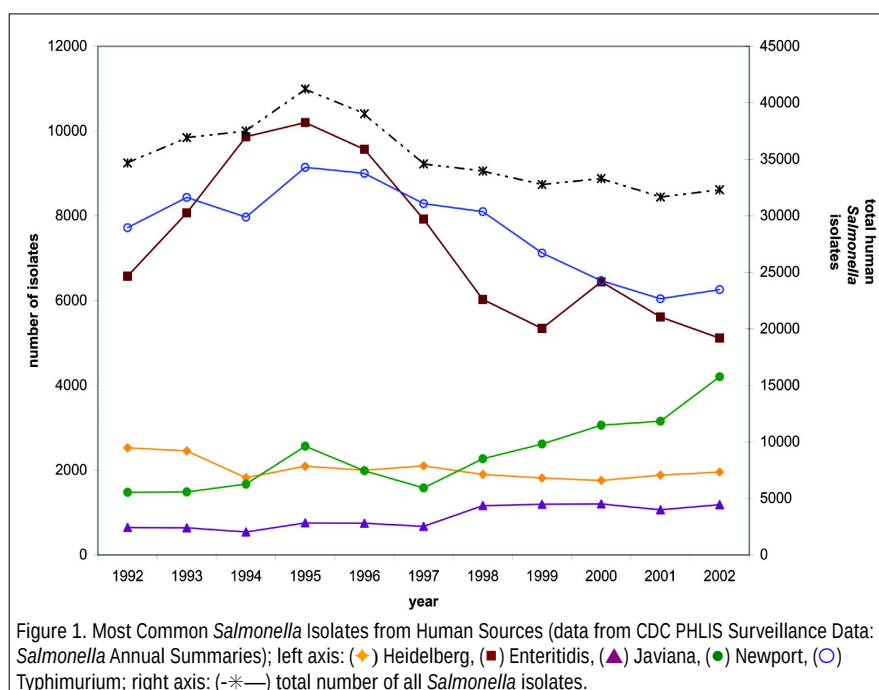
There are three main ways *Salmonella* can enter the food supply to cause illness. Animals harbor *Salmonella*, making meats, poultry, eggs, and milk often implicated vehicles. *Salmonella*, which are introduced into the environment, possibly through manure and litter, may persist and contaminate fruits and vegetables on the farm. Cross-contamination in the food service environment or the home, often between raw poultry and ready-to-eat (RTE) products, such as raw vegetables, can also cause salmonellosis.

Some *Salmonella* are host-adapted (e.g., *Salmonella pullorum* in poultry, *Salmonella dublin* in cattle); however, most *Salmonella* are not. Although any *Salmonella* is a potential pathogen for humans, most foodborne salmonellosis is caused by non-host-adapted serotypes.

Much human salmonellosis is directly related to human association with animals, both wild and domestic. Foods of animal origin are vehicles for salmonellosis. *Salmonella* was isolated in 19–54% of cattle carcasses, 1.9% of beef samples at retail and 4.2% of retail chicken samples (Beach et al., 2002; Zhao et al., 2001). In a review of *Salmonella* in meats and poultry, Silliker and Gabis (1986) introduced the problem as follows:

“The animal-to-man link is only one factor in the epidemiology of human salmonellosis. Contaminated red meat and poultry provide a nidus (source of infection) for *Salmonella*, which man nurtures through mishandling. Furthermore, inedible parts of the animal are processed to yield important components of livestock feeds. As a result of poor manufacturing practices (post-processing contamination), these rendered animal by-products become recontaminated with *Salmonella*, which, in turn, are carried into the feeds. The consumption of these feeds by livestock, followed by animal-to-animal transmission, completes the *Salmonella* cycle.

This is not to suggest, however, that contaminated feeds are the only source of *Salmonella* in livestock. Epidemiological evidence indicates that there is a direct



link between the presence of *Salmonella* in meat and poultry and human salmonellosis. Man induces salmonellosis through improper food handling practices and perpetuates salmonellosis through recontamination of rendered animal by-products, which are incorporated into livestock feeds.”

Historically, egg products (dried, frozen or liquid eggs, with or without added ingredients, as defined in the Egg Products Inspection Act; U.S. Congress, 1970) were a significant source of human salmonellosis in the United States. In 1990, the U.S. Dept. of Agriculture (USDA) required that eggs produced by flocks previously implicated in human disease be tested for *Salmonella* (FDA/CFSAN, 2003b). Mandatory pasteurization of egg products was responsible for greatly reducing eggs as a major cause of salmonellosis, and consumer awareness of the need to refrigerate eggs also contributed in the decreased incidence of disease. However, since 1999, the incidence of *Salmonella* Enteritidis has remained stable. The microorganism is localized inside eggs, making thorough cooking imperative. The CDC estimates that 75% of *Salmonella* Enteritidis cases result from the consumption of raw or undercooked grade A whole-shell eggs (FDA/CFSAN, 2003b). This serotype was the second most commonly reported human serotype to the CDC in 2001. A portion of these illnesses occurred as a result of pooling raw shell eggs in institutional or foodservice settings. The potential for temperature abuse or long holding times, combined with the possibility of inadequate cooking, resulted in the recommendation that institutions use pasteurized egg products or pasteurized in-shell eggs instead of raw-shell eggs (CDC, 2003c).

Consumption of raw milk may also cause human salmonellosis. In one study, *Salmonella* was isolated in 6.1% of bulk raw milk samples (Jayarao and Henning, 2001). Between 1972–2000, 16 outbreaks of salmonellosis resulted from raw milk consumption (CDC, 2003a). Milk-borne salmonellosis was particularly prevalent in Scotland, where, prior to 1983, the sale and consumption of raw milk was common (Sharp, 1986). Milk outbreaks in England and Wales were primarily associated with raw milk. The sale of raw milk is legal in 27 states, although many have restrictions (CDC, 2003a; The Weston A. Price Foundation, 2003). Pasteuriza-

tion of milk destroys *Salmonella* and currently is the only effective means of control for milk. However, inadequate pasteurization or contact with raw milk after pasteurization can result in contaminated milk. In one pasteurized milk outbreak in 1985, there were more than 16,000 laboratory-confirmed cases of salmonellosis and several deaths (Anonymous, 1986).

Animal products are not the only sources of human salmonellosis. Produce can serve as a vehicle of *Salmonella* as well, becoming contaminated either on the farm or through cross-contamination with contaminated products. Consumption of produce contaminated with *Salmonella* has recently caused several outbreaks. In 2000, 361 individuals in England and Wales acquired salmonellosis after eating lettuce, and although the lettuce appeared to come from one of three farms, investigators could not conclusively find the source of contamination (Horby et al., 2003). The strain, *S. enterica* subsp. *enterica* serotype Typhimurium DT 104, is resistant to several antibiotics, and the increase in its prevalence poses challenges in treatment of the infection. Raw sprouts, particularly alfalfa and mung bean, have been involved in 15 salmonellosis outbreaks since 1995 (Thomas et al., 2003). Seeds may be contaminated, and the warm, moist conditions for sprouting allow growth of the pathogen. On-farm contamination of cantaloupes resulted in 24 cases of salmonellosis in 1997 (Mohle-Boetani et al., 1999). Contamination of cantaloupes on one farm caused multistate outbreaks of salmonellosis each spring between 2000 and 2002 (CDC, 2002b). Since produce may be eaten raw, different control measures are necessary to prevent illness when the pathogen is introduced on the farm.

While *Salmonella* may survive in contaminated foods as a result of improper cooking, it is more common that cross-contamination of foods after cooking is the source of *Salmonella*. Foodservice workers or in-home food preparers may transfer salmonellae from raw products to cooked or other uncontaminated foods as a result of unsanitary preparation practices (e.g., failure to wash hands) between handling of these foods. *Salmonella* can also be transferred from contaminated raw foods to equipment surfaces, such as knives, cutting boards, and counter tops, and then from equipment to previously

uncontaminated foods. Once contamination occurs, the situation may be further complicated by improper storage of the product before serving (e.g., kept at room temperature, improperly refrigerated, or held in warmers within the growth range for *Salmonella*).

Although responsible for fewer outbreaks, contamination of foods by infected workers cannot be ignored as a cause of foodborne salmonellosis. Some infected individuals may excrete *Salmonella* for weeks, months, and, occasionally, years with little or no evidence of disease. Improper hygiene practices by these individuals may lead to either contamination of foods or direct person-to-person contamination.

Control Measures

Different control measures exist depending on the mode of contamination of the food. Reduction of the incidence of *Salmonella* contamination of foods requires a number of approaches to the problem, beginning at the farm and going right through to the kitchen.

Several approaches have been taken to reduce the carriage of *Salmonella* by animals. Vaccination of laying chickens significantly reduced the percent of eggs positive for *Salmonella* Enteritidis (Woodward et al., 2002). Other research has examined the effect of probiotics on the intestinal microflora of chickens. In this approach, the goal is to colonize the chicken with known microorganisms to “competitively exclude” *Salmonella*. Successful reduction in the percent of the flock positive for *Salmonella* was achieved when the feed was supplemented with yeast (Line et al., 1998) or when chickens were both sprayed with a patented mucosal starter culture and fed the culture through water (Bailey et al., 2000).

In July 1996, USDA's Food Safety and Inspection Service (FSIS) published pathogen reduction performance standards for *Salmonella*. The performance standard (percent positive) was established by collecting the average baseline in the industry. Companies must use HACCP and other measures to ensure that the percent of their product that is positive for *Salmonella* falls below this baseline. The performance standard has been expanded to include steers, cows, broilers, hogs, and several related products (FSIS, 1998). Between 1997 and 2003, FSIS reported a 66% decrease in the presence of *Salmonella* in raw meat

Scientific Status Summary

and poultry (USDA, 2003). Due to the difficulty in completely eradicating *Salmonella* from poultry, irradiation was approved in 1999 as a means to control the microorganism as part of a HACCP system (USDA, 1999).

The most common method of eliminating *Salmonella* from food products is heat processing. *Salmonella* is heat sensitive, and ordinary pasteurization or cooking conditions are generally sufficient to kill it in high-moisture foods. As with other microorganisms, the heat resistance of *Salmonella* is markedly increased as water activity (a_w) decreases. Current U.S. standards for milk pasteurization result in a more than 10^{12} -fold reduction of viable *Salmonella* found in milk (Read et al., 1968). Minimum heat processes for pasteurization or cooking of most high-moisture products will eliminate *Salmonella* from these products. Occurrence of *Salmonella* in such processed products generally results from post-processing contamination.

In some cases, dry products may be heat processed for the purpose of elimination of *Salmonella*. Ayres and Slosberg (1949) and Banwart and Ayres (1956) demonstrated the effectiveness of dry-heat treatment on reducing *Salmonella* in pan-dried egg white. This work led to the eventual requirement for dry-heat pasteurization of this product (U.S. Congress, 1970). Similar treatments have been applied to gelatin, rendered animal by-products, nonfat dry milk, and chocolate.

Other than heat, the major manufacturing factors responsible for elimination of *Salmonella* from food products are acidification and reduction of a_w . Goepfert and Chung (1970) investigated the fate of *Salmonella* during sausage fermentation and concluded that the combination of acidity and sodium chloride was the principal reason for the demise of *Salmonella* during fermentation. Similarly, Smittle (1977) reviewed the literature on survival of *Salmonella* in mayonnaise and salad dressing and concluded that the principal factor responsible for the death of salmonellae in these products is acidity, but that a second important factor is reduction of a_w as affected by moisture, NaCl, and sugar concentration. These same factors are responsible for control of *Salmonella* in a variety of fermented dairy, meat, and vegetable products.

Salmonella may survive for extended periods in dehydrated foods. However,

some death occurs during dehydrated storage, depending on the relative humidity (or a_w) and storage atmosphere. The rate of death of *Salmonella* in food preserved by reduced a_w is increased at higher a_w levels, temperatures, and oxygen levels (Genigeorgis and Riemann, 1979). In low-moisture products such as peanut butter and chocolate, *Salmonella* may remain for years, with little loss of viability.

Moist, perishable products are generally distributed under refrigerated or frozen conditions. Although freezing and frozen storage can have some lethal effects on *Salmonella*, it is well recognized that *Salmonella* remain viable for long periods of time in frozen foods and that survival is enhanced as the storage temperature decreases (Georgala and Hurst, 1963).

The presence of *Salmonella* in certain types of produce seems to result from introduction of the pathogen on the farm. Contamination in these products, which are typically not heated before consumption, presents challenges to the food industry (CDC, 2002b; Thomas et al., 2003). For sprouts, chemical disinfection of the seed coat is not mandatory, but is recommended by the Food and Drug Administration (FDA). A survey of sprout producers in California found that most alfalfa sprout growers followed FDA recommendations. Mung bean producers were less apt to follow the guidance (Thomas et al., 2003). An outbreak involving 87 cases of *Salmonella mbandaka* was linked back to growers who did not disinfect seeds (Gill et al., 2003). The recommended level of chlorine treatment, 20,000 ppm, still may not be sufficient to eliminate *Salmonella*, especially if they are localized inside the sprout tissue, as Gandhi et al. (2001) observed. Therefore, compliance on the part of the grower, while worthwhile, does not guarantee a *Salmonella*-free product since chemical sanitizers may not be wholly sufficient to eliminate the presence of *Salmonella* in fruits and vegetables with natural openings and crevices (Beuchat and Ryu, 1997).

When purchasing items likely to be contaminated with *Salmonella*, such as chicken, there are steps the food handler can take to prevent salmonellosis from occurring in the home or food service environments. These general food safety practices include avoiding cross-contamination, thoroughly cooking foods,

and storing foods at the right temperatures.

Shigella

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Shigellosis, or bacillary dysentery, as it is commonly known, is caused by bacteria of the genus *Shigella*, which include *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei* (Figure 2) (Bryan, 1979). The normal habitat for *Shigella* is the intestinal tract of humans and other primates.

Significance as a Pathogen

Data suggest that *Shigella* is responsible for less than 10% of reported foodborne illnesses per year, infecting approximately 300,000–450,000 people annually (FDA/CFSAN 2003b; Mead et al., 1999). Although the primary mode of transmission appears to be person-to-person by the fecal-oral route (Feldman and Riley, 1985), there have been some documented cases of foodborne shigellosis. The main source of *Shigella* involved in outbreaks is asymptomatic carriers or persons recovering from disease. *Shigella* may persist in the intestinal tract for months (Bryan, 1978).

Symptoms of shigellosis include diarrhea, abdominal pain, fever, and vomiting. The severity of the disease may vary from very mild to severe diarrhea with bloody stools, mucus secretions, and dehydration. Reactive arthritis, hemolytic uremic syndrome and Reiter's disease are sequelae associated with shigellosis (FDA/CFSAN, 2003b). Generally, foodborne shigellosis is characterized by a high attack rate, common-source epidemiology, and short incubation periods of 12–50 hr (FDA/CFSAN, 2003b). Symptoms usually persist 3–14 days. Infection by some strains results in a 10–15% mortality rate. Frequently, an asymptomatic carrier state may develop during convalescence, lasting from a few days to several months. Human-volunteer studies indicate that ingestion of as few as 10–100 microorganisms can induce illness (Morris, 1986).

The disease is caused by invasion of the intestinal mucosa. Enterotoxins, classically referred to as Shiga toxins, may be produced by *S. dysenteriae* and possibly by *S. flexneri* type 2A (Keusch et al., 1985). Shiga toxins block mRNA transcription and induce apoptosis in endothelial cells (Erwert et al., 2003; Thorpe et al., 1999).

Secondary infections with *Shigella*

occur frequently. Shigellosis can be a major problem in daycare centers, and control is difficult due to the low infectious dose, unpredictable acquisition of antimicrobial resistance, frequency of mild infections and carrier status, and the frequency with which young children are transferred from one daycare center to another (CDC, 1984). The disease is disproportionately common among the urban poor, immigrants, Native Americans, daycare centers, and institutions (Bryan, 1978; Morris, 1986). FoodNet data from 2003 captured 3,875 confirmed cases of shigellosis within the 13% of the population monitored. Extrapolated, this suggests almost 30,000 cases would be confirmed in the U.S. population (CDC, 2003b). In the early 1900s, *S. dysenteriae* and *S. flexneri* predominated (Bryan, 1978). The pattern of isolations suggests that prior to 1945, direct fecal contamination of food and water supplies may have been the primary vector of disease. As socioeconomic and environmental sanitation (municipal sewage disposal, safe water supplies, and food hygiene) have improved, the relative prevalence of *S. sonnei* has increased. The explanation for this phenomenon is not clear, but it may result from differences among the shigella in their ability to survive and grow outside their human and animal hosts.

Association with Foods

An estimated 20% of the total number of cases of shigellosis involve food as the vehicle of transmission (Mead et al., 1999). The principal foods involved in outbreaks include a variety of salads and seafoods (Morris, 1986) contaminated during handling by infected workers (Bryan, 1978). Six separately reported outbreaks in the United States and Canada in 1998 involving *S. sonnei* were traced back to parsley grown in Mexico (Naimi et al., 2003). The presence of *S. sonnei* in commercially produced dip caused an outbreak on the Pacific Coast in 2000. The product, marketed under several brand names, was ultimately recalled (CDC, 2000c). Improper refrigeration of the contaminated product often contributes to illness. After the initial infection from a contaminated food, the disease readily spreads from person to person by the fecal-oral route of transmission.

Shigella usually are considered to be relatively fragile; i.e., they do not survive well outside the host (Bryan, 1978). Like

most other members of the family *Enterobacteriaceae*, they are readily killed by most heat treatments employed in the processing and preparation of foods, and do not survive well at pH below 4.5. However, studies on the survival of shigella suggest that under certain selected conditions, they can survive for extended periods in foods (Bryan, 1978). For example, *S. sonnei* and *S. flexneri* have been reported to survive at 25°C (77°F) in flour and in pasteurized whole milk for more than 170 days; in eggs, clams, and shrimp for more than 50 days; in oysters for more than 30 days; and in egg whites for more than 20 days. At lower temperatures, such as 20°C (68°F) and 0.5°C (32.9°F), survival for longer periods has been reported (Taylor and Nakamura, 1964).

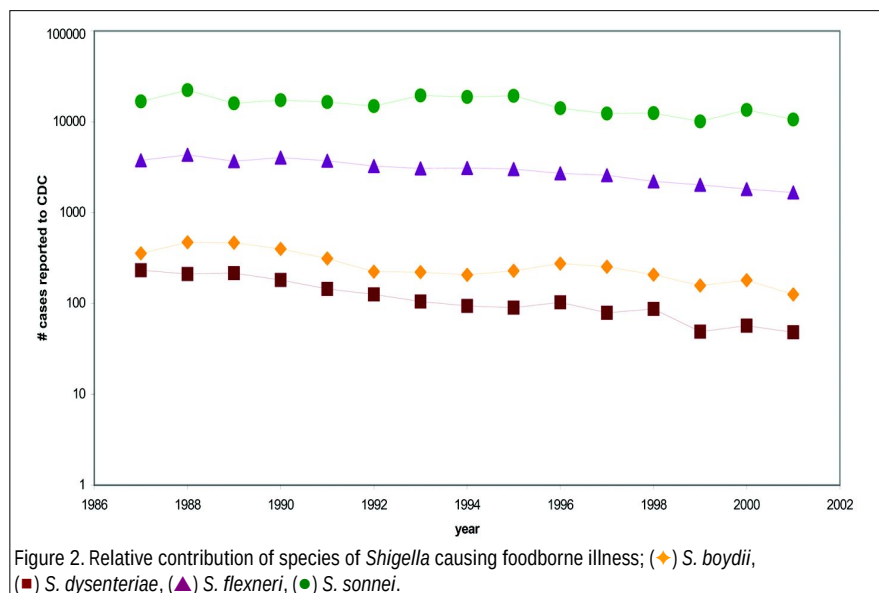
These laboratory data suggest that shigella may survive for extended periods in foods and may grow under selected conditions. However, in practice, *Shigella* is rarely isolated from processed products. Manufacturers do not routinely test their products, raw materials, or processing environments for *Shigella*, and there is no evidence to suggest that routine testing is warranted. Most outbreaks result from contamination of raw or previously cooked foods during preparation in the home or in foodservice establishments. Generally, the source of contamination can be traced to a carrier whose personal hygiene is poor (Bryan, 1979). In underdeveloped countries, harvesting of seafood from fecally contaminated waters and use of unsanitary water in food preparation may be sources of disease.

Control Measures

Shigellosis is a significant cause of foodborne illness in both developed and developing countries. Food microbiologists should be aware that *Shigella* species can cause a rather severe form of foodborne illness (Smith, 1987) and that relatively low numbers of the microorganisms can cause disease. Products suspected of foodborne illness should be analyzed for *Shigella*.

Further studies are needed to determine the roles of the various toxins and virulence factors in the disease process. The epidemiology of shigellosis should be reevaluated, with particular emphasis on the environmental factors associated with the increasing incidence of *S. sonnei* and *S. flexneri*. The waning incidence of *S. dysenteriae* in U.S. epidemiological data clearly indicate that the major cause of shigellosis in developed countries is infected food handlers. Thus, prevention and control of shigellosis requires either that infected humans not be permitted to handle foods or that they practice good personal hygiene such that, even if infected, the food does not become contaminated. Certainly, exclusion of infected individuals from food handling is desirable. However, routine testing of food workers for *Shigella* is neither practical nor necessary.

The principles necessary for control of *Shigella* at the preparation and service level are the same as those necessary for control of other more widely recognized pathogens such as *Salmonella* and *Campylobacter*. The increasing awareness of consumers and employees of foodservice establishments of the po-



Scientific Status Summary

tential for foodborne diseases should do much to reduce the incidence of shigellosis in the developed countries. In the developing countries, control of shigellosis, as always, will depend on improved hygiene and waste-handling practices.

Campylobacter jejuni and other species

Michael P. Doyle

Originally considered a pathogen principally of veterinary significance, *Campylobacter jejuni* (formerly known as *Vibrio fetus*) was known to cause abortion in sheep. Other enteric campylobacters that may be transmitted through food include *C. coli*, *C. lari*, and *C. upsaliensis*, although many other species have been identified.

Significance as a Pathogen

Following the development of procedures for detecting campylobacters in stool specimens, *C. jejuni* became recognized as a leading cause of acute bacterial gastroenteritis in humans. Recent evidence suggests that *Campylobacter* causes 2–4 million cases per year in the United States (FDA/CFSAN, 2003b; Mead et al., 1999). Common symptoms of *Campylobacter* enteritis include profuse watery or sticky diarrhea (sometimes containing blood), abdominal cramps, headaches, muscle pain, and nausea. The mortality rate is low (0.1%) and often occurs in susceptible populations such as the elderly, young, or immunocompromised (FDA/CFSAN, 2003b). Roughly 25% of cases experience relapses. Although sequelae such as Guillain-Barré syndrome, meningitis, recurrent colitis and acute cholecystitis are uncommon, campylobacter is associated with more than 30% of cases of Guillain-Barré (FDA/CFSAN, 2003b; Mead et al., 1999). Human volunteer and retrospective studies of food-associated outbreaks of *Campylobacter* enteritis revealed that ingesting relatively small numbers (only a few hundred cells) of *C. jejuni* can produce illness. Symptoms manifest after an incubation period of two to five days, and generally last 7–10 days. Treatment with erythromycin can decrease an individual's duration of shedding the microorganism (FDA/CFSAN, 2003b).

Association with Foods

Campylobacters are harmless inhabitants of the gastrointestinal tract of a variety of wild and domestic animals. Studies have revealed that as many as 30–100% of poultry, 40–68% of cattle, and up to 76% of swine carry *C. jejuni* or *C. coli* in their intestinal tracts (Beach et al., 2002; Harvey et al., 1999). For this reason, the microorganism is often associated with unprocessed foods of animal origin. Surveys of U.S. retail fresh red meat and poultry show that 12–35% of turkey, 64% of chicken, 2–5% of pork, 0–5% of beef, 8% of lamb and 9% bulk tank raw milk contained *C. jejuni* and/or *C. coli* (Jayarao and Henning, 2001; Logue et al., 2003; Stern et al., 1985; Zhao et al., 2001). It is estimated that roughly half of all cases of *Campylobacter* enteritis are associated with undercooked chicken or cross-contamination with raw chicken (FDA/CFSAN, 2003b). In a 1996 outbreak, raw lettuce served as the vehicle for *Campylobacter* infections, presumably after contact with raw chicken (CDC, 1998). Other foods implicated as vehicles of outbreaks include raw milk, raw beef, clams, and cake (likely contaminated by a *C. jejuni*-infected food handler). Consumption of *C. jejuni*-contaminated raw milk from an organic dairy farm made 75 Wisconsin residents ill in 2001 (CDC, 2002c). Incompletely pasteurized milk was responsible for an outbreak of *Campylobacter* enteritis affecting 110 people in the United Kingdom (Fahey, 1995). Although *C. jejuni* is responsible for more foodborne illness than *C. coli*, the significance of the latter should not be overlooked. Estimates from studies in England and Wales revealed that approximately 25,000 cases of foodborne illness were caused by *C. coli* in the year 2000 (Tam et al., 2003). Outbreaks of *Campylobacter* enteritis are generally small; however, untreated surface water used in municipal water supplies has been responsible for large outbreaks involving thousands of individuals.

The principal route by which *C. jejuni* contaminates food is through fecal contamination by *Campylobacter*-infected carriers. Raw meats and poultry become contaminated during processing when intestinal contents contact meat surfaces. Raw milk may be contaminated by contact with bovine feces or pos-

sibly by a mastitic infection of the bovine udder. Fresh produce can be contaminated by fecal-laden irrigation water, infected harvesters with poor hygienic practices, or tainted processing water. Although eggs have rarely been linked to outbreaks of *Campylobacter* enteritis, *C. jejuni* has been isolated from the surface of eggs laid by *Campylobacter*-infected hens (Doyle, 1984; Finch and Blake, 1985).

C. jejuni and other campylobacters are not likely to grow in foods that remain edible because the organisms: (1) will not grow below 30°C (86°F); (2) require microaerophilic (low concentrations of oxygen, ideally 5–10%) conditions for growth; (3) grow slowly even under optimal conditions; and (4) are not good competitors with other microflora. Additionally, *C. jejuni* is a very fragile microorganism, being sensitive to drying, normal atmospheric concentrations of oxygen, storage at room temperature, acidic conditions, disinfectants, and heat. Because the microorganism is quite sensitive to stressful environmental conditions when outside of the host's digestive tract, campylobacters are not likely to be a problem in processed foods that receive a pasteurization treatment or are dehydrated. However, the microorganism is often associated with and transmitted by raw, refrigerated foods of animal origin.

Control Measures

C. jejuni infections can be prevented by properly pasteurizing or cooking foods (especially foods of animal origin) and avoiding cross-contamination of cooked or RTE foods by utensils, equipment, or cutting surfaces that are not properly cleaned and disinfected after contact with fresh, uncooked meats and foods likely to be contaminated with the microorganism. Pasteurization will eliminate viable campylobacters in milk. Use of potable irrigation and processing water and safe food handling practices by harvesters are important to the safety of produce. Although *C. jejuni* does not survive well in foods, refrigeration prolongs survival. Freezing food will substantially reduce the initial *Campylobacter* population; however, a small number of survivors may remain viable for many months (Moorhead and Dykes, 2002). Testing foods for the pres-

ence of *Campylobacter* is difficult since enrichment with antibiotics is necessary and cells must be grown in an environment of 5% O₂ and 10% CO₂.

Yersinia enterocolitica

Michael P. Doyle

Yersinia enterocolitica is not a frequent cause of human infection in the United States; illness is more common in Northern Europe, Scandinavia, and Japan.

Significance as a Pathogen

Although CDC estimates that there are 17,000 cases of yersiniosis, the infection caused by *Y. enterocolitica*, per year, less than 7,500 total cases were reported between 1990 and 1999 (Ackers et al., 2000; FDA/CFSAN, 2003b). When the microorganism is involved in illness, which typically manifests 24–48 hr after ingestion, the symptoms can be quite severe; but fatalities are rare. The infective dose is unknown. Yersiniosis occurs most commonly in the form of gastroenteritis. Children are most severely affected with symptoms of intense abdominal pain (often mimicking appendicitis), diarrhea, fever, and vomiting. Symptoms of pseudoappendicitis have resulted in many unnecessary appendectomies in children. The illness has also been misdiagnosed as Crohn's Disease (FDA/CFSAN, 2003b). Fatality from gastroenteritis is rare, and recovery is generally complete within one or two days. Arthritis has been identified as an infrequent but significant sequela in 2–3% of *Y. enterocolitica* infection cases. Other syndromes may include mesenteric lymphadenitis, terminal ileitis, erythema nodosum, septicemia, and meningitis.

Association with Foods

Y. enterocolitica is principally a zoonotic bacterium that has been isolated from a variety of animals. However, most animal isolates, with the exception of swine isolates, are not human pathogens. Swine is an important and the principal reservoir for virulent strains of *Y. enterocolitica*, with such isolates often recovered from the tonsils and tongues of apparently healthy animals (Doyle et al., 1981). A study in Japan revealed that rats in slaughterhouse areas may also be

carriers of virulent types of *Yersinia* (Zen-Yoji et al., 1974).

Not surprisingly, because swine are the primary reservoir of virulent types of *Y. enterocolitica*, illness has been associated with the consumption of pork intestines. The product, known as chitterlings, is traditionally eaten during the winter holidays, generally by African Americans, and the incidence of yersiniosis increases in December for the consuming population. Infants and children are most often affected, largely because of caretakers who do not properly wash their hands between preparing chitterlings and feeding young children, or practice adequate kitchen hygiene (Jones et al., 2003; Lee et al., 1991). For epidemiological purposes, these cases are not reported as foodborne illness, since the food is not ingested, but clearly

***Y. enterocolitica* is commonly present in foods; however, with the exception of pork, most isolates from foods are avirulent.**

the porcine intestines served as the source of the pathogen.

A study of bulk tank milk revealed that 6.1% of samples were positive for *Y. enterocolitica*. Further testing determined that all strains were virulent (Jayarao and Henning, 2001). Chocolate milk, pasteurized milk, and tofu packed in unchlorinated spring water have all been vehicles of outbreaks of yersiniosis (Ackers et al., 2000). The precise mode of transmission was not determined, but in each situation there was thought to be a lack of sanitary practice or deficiencies in good manufacturing practices. Circumstantial evidence suggests that porcine waste may have been the original source of *Y. enterocolitica* involved in some of the food-associated outbreaks (Toma and Deidrick, 1975; Wauters, 1979).

Y. enterocolitica is commonly present in foods; however, with the exception of pork, most isolates from foods are avirulent. The infrequent presence of

pathogenic strains in foods may explain why there are relatively few reported food-associated outbreaks of yersiniosis. The microorganism is one of a few foodborne pathogens that can grow at refrigeration temperatures. Studies in raw pork revealed that within 10 days at 7°C (44.5°F) the microorganism could grow from a few hundred cells to millions/g (Hanna et al., 1977). Hence, refrigeration, which traditionally has been an important means of controlling the growth of foodborne pathogens, is not an effective method for controlling *Yersinia*.

Control Measures

Y. enterocolitica is sensitive to heat (e.g., pasteurization at 71.8°C for 18 sec), sodium chloride (5%), and high acidity (pH 4.0), and will generally be inactivated by environmental conditions that will kill *Salmonella* (Robins-Browne, 1997). However, since *Yersinia* will grow at refrigeration temperatures, cold storage can be a means of selectively promoting growth of the microorganism in foods. Therefore, it is important to eliminate the microorganism from foods (especially pork, milk, and foods that may have direct or indirect contact with porcine waste) by pasteurization or cooking. Care should be taken to avoid cross-contamination of processed, RTE foods with pork and porcine wastes, as well as animal and human fecal contamination.

***Listeria monocytogenes* and other *Listeria* species**

Originally authored by Joseph Lovett and Robert M. Twedt. Reviewed by Stephanie Doores.

Prior to the 1980s, listeriosis, the disease caused predominantly by *Listeria monocytogenes*, was primarily of veterinary concern, where it was associated with abortions and encephalitis in sheep and cattle. Interestingly, evidence indicates that veterinary listeriosis was frequently foodborne. As a result of its wide distribution in the environment, its ability to survive for long periods of time under adverse conditions, and its ability to grow at temperatures as low as –0.4°C, *Listeria* has since become recognized as an important foodborne pathogen (ICMSF, 1996).

Significance as a Pathogen

Immunocompromised humans are highly susceptible to virulent *Listeria*. The microorganism causes approximately 1,600 cases of listeriosis, resulting in approximately 415 deaths annually (FDA/CFSAN, 2003b). Only the hemolytic species of *Listeria* (*L. monocytogenes*, *L. seeligeri*, and *L. ivanovii*) are associated with pathogenicity. Of these, only *L. monocytogenes* is consistently pathogenic; *L. ivanovii* has rarely been reported to be involved in human pathology, and *L. seeligeri* was reported only once to be the cause of meningitis in a non-immunocompromised adult.

Listeriosis is a very serious and often fatal infection primarily affecting the elderly and perinates. However, gastrointestinal illness has recently been recognized as a possible manifestation of ingestion of *Listeria*. Unlike the more severe form of listeriosis, gastrointestinal illness, which generally results more than 12 hr after ingestion, primarily affects seemingly healthy adults. In some instances, this type of illness is associated with the consumption of large doses of *L. monocytogenes* (Dalton et al., 1997).

In humans, ingestion of as few as 1,000 cells of the bacteria, which then invade macrophages, may be marked by a flu-like illness (malaise, diarrhea, and mild fever). This enteric phase, however, may be without symptoms, or so mild as to go unnoticed. A carrier state may develop. Between 1–10% of symptomless persons may be excretors of *L. monocytogenes* (FDA/CFSAN, 2003b). Identification of *Listeria* as the causative agent of foodborne illness is complicated by the high carrier rate in humans and must be isolated from the food for confirmation (FDA/CFSAN, 2003b).

Following invasion of macrophages, virulent strains of *Listeria* may then multiply, resulting in disruption of these cells and septicemia. In this stage, occurring five days to three weeks after ingestion, the microorganism has access to all body areas and may involve the central nervous system, the heart, the eyes, or other locations, including the fetuses of pregnant women (FDA/CFSAN, 2003b). The stage of pregnancy upon exposure of the fetus to the microorganism determines the outcome for the fetus (abortion, stillbirth, or neonatal sepsis). The perinatal and neonatal mortality rate is 80% (FDA/CFSAN, 2003b). Death is rare in healthy adults; however, the mortality rate may approximate 50% in the

immunocompromised, newborn, or very young (FDA/CFSAN, 2003b). Fever is a common symptom, and other complaints may vary from nonspecific fatigue and malaise to enteric symptoms.

Forms of listeriosis involving the central nervous system include meningitis, encephalitis, and abscesses. The clinical course for meningitis develops and progresses suddenly, and the fatality rate can be as high as 70%. Localized forms of listeriosis, which are rare, include endocarditis, endophthalmitis, and osteomyelitis, with the involvement of other sites such as skin, spleen, gall bladder, and lymph nodes. All forms of listeriosis are more likely to accompany immuno-

The first reported outbreak of foodborne listeriosis occurred between March and September 1981.

compromised states, whether natural (as in pregnancy, or in the elderly) or induced as a result of medical treatment (such as with corticosteroids). Individuals using antacids or cimetidine may also be more susceptible to infection, because of changes in stomach acidity. Listeriosis is often treated with penicillin or ampicillin (FDA/CFSAN, 2003b).

Association with Foods

Listeria is ubiquitous in nature, occurring in soil, vegetation, and water (Beuchat and Ryu, 1997; Coyle et al., 1984; Pearson, 1970), and therefore is frequently carried by humans and animals. *Listeria* can survive for long periods in both soil and plant materials. Ingestion of contaminated silage by ruminants has been linked to the occurrence of *Listeria* in milk (Donnelly, 1987).

Biological oxidation during wastewater treatment favors the growth of *Listeria* in sewage. Sludge provides a favorable environment for survival and growth of *L. monocytogenes* for many months (Guenich and Muller, 1984). *L. monocytogenes* in concentrations of 10^3 – 10^4 cells/mL have been found associated with sewage plant effluents, while sewage sludges have shown higher concentrations. This makes the use of sewage plant effluent or waters receiving sewage plant effluent for irrigation of edible

crops dangerous. The use of sludge for fertilization of edible crops is equally hazardous.

L. monocytogenes can grow in the pH range of 4.39–9.4 in a good growth medium (ICMSF, 1996). Environments with pH values outside that range are unfavorable for survival. The microorganism has readily survived the pH 5 environments of cottage cheese and ripening Cheddar cheese, and was detectable in the latter product (pH 5.0–5.15) for more than one year (Ryser and Marth, 1987).

Listeria is salt tolerant, growing to levels of visible turbidity in the laboratory media Tryptic Soy Broth (pH 5.0) after 41 hr at 25°C in presence of 10% sodium chloride (ICMSF, 1996). However, 12.5% sodium chloride inhibited the growth of *L. monocytogenes* at pH 6.0 between 8 and 30°C (Razavilar and Genigeorgis, 1998). *Listeria* is reported to survive for three months in dry fodder and more than six months in dry straw. Dry feces have been reported to maintain viability of the microorganism for more than two years (Gray, 1963), and survival in soil for up to 295 days has been recorded. *Listeria* is relatively resistant to drying, as demonstrated on glass beads and tile surfaces. Survival is temperature dependent, with lower temperatures enhancing survival (Dickgieber, 1980; Welshimer, 1960), an important factor in *Listeria*'s significance in the food chain.

The first reported outbreak of foodborne listeriosis occurred between March and September 1981. Coleslaw was implicated as the cause of 34 cases of perinatal listeriosis and seven cases of adult listeriosis in the Maritime Province of Nova Scotia. Perinatal cases were characterized by acute febrile illness in pregnant women, followed by spontaneous abortion (five cases), stillbirth (four cases), live birth of a seriously ill premature or term infant (23 cases), or live birth of a well infant (two cases). The fatality rate for infants born alive was 27%. The outbreak strain was isolated from two unopened packages of coleslaw from the plant but was not cultured from the manufacturing plant environment. The incriminated coleslaw was traced to a farm where the cabbage was grown in fields fertilized with sheep manure. Two of the sheep on the farm had previously died from listeriosis (Schlech et al., 1983).

Although listeriosis is more com-

monly reported as sporadic cases, of which about 20% result from the consumption of unheated hot dogs and undercooked chicken, there have been many outbreaks involving a variety of foods (FDA/CFSAN and USDA/FSIS, 2003). Hot dogs and deli meats contaminated with *L. monocytogenes* serotype 4b caused a 1998–1999 outbreak affecting 101 individuals in 22 states (FDA/CFSAN and USDA/FSIS, 2003). In 2002, 63 cases of listeriosis, of which three were perinatal, resulted from the consumption of sliced deli meat in eight Northeastern states. This type of food product was also responsible for an outbreak in 2000 affecting 29 people in 10 states (CDC, 2000a).

The soft cheeses are now infamous for their susceptibility to contamination with *L. monocytogenes*, presumably due to the use of raw milk or because of post-pasteurization contamination. Not only is the manufacturing process open to contamination, but the cheese can serve as a growth environment where *L. monocytogenes* can multiply during the storage period, even under refrigeration at 4°C (39.2°F). Concentrations of *Listeria* as great as 10⁶ cfu/g have been noted. Mexican-style cheese made with raw milk was implicated in two separate outbreaks, one in 1984 causing 142 illnesses, and one in 2000–2001 resulting in 12 cases. The mortality rate was 33.8% and 41.7%, respectively (CDC, 2001; Linnan et al., 1988).

While association of listeriosis with raw milk consumption is not rare, it is infrequent and sporadic. The results of surveys of raw milk for *L. monocytogenes* show that the presence of the microorganism in raw milk is common in the United States and Europe (Dominguez Rodriguez et al., 1985; Lovett et al., 1987). The 2003 risk assessment conducted jointly by FDA/CFSAN and USDA/FSIS pooled the data of 45 studies, which showed an overall positive rate of 4.1% (FDA/CFSAN and USDA/FSIS, 2003). The presence of *L. monocytogenes* in pasteurized milk in the United States is rare, with an average percent positive of 0.4% in more than 10,000 samples tested in 30 studies conducted worldwide (FDA/CFSAN and USDA/FSIS, 2003).

Control Measures

Because of the severity of listeriosis and the ability of the microorganism to grow at refrigeration temperatures, both

the FDA and USDA have a “zero tolerance” (absence in 25 g) for *L. monocytogenes* in RTE foods. The 2003 risk assessment prepared jointly by the agencies estimated the relative risk of listeriosis in 23 categories of RTE foods. The agencies report that the results of the risk assessment will be used to focus control efforts on high-risk foods (FDA/CFSAN and USDA/FSIS, 2003). In terms of risk per serving, deli meats ranked as having the highest risk.

Because of the link between dairy foods, particularly milk, and *L. monocytogenes*, the adequacy of pasteurization temperatures and times to inactivate *L. monocytogenes* were intensively studied. Scientific consensus on this subject holds that the risk of post-pasteurization contamination is a far more serious threat than survival of the microorganism to pasteurization heat treatment (63°C for 30 min or 71.7°C for 15 sec). Two studies by Bunning et al. (1986, 1988) failed to support the hypothesis that an intracellular location of the bacteria in polymorphonuclear leucocytes may confer heat resistance and allow survival during pasteurization. Heat shock at 42 or 48°C for up to 60 min did not confer significantly increased thermotolerance at 52 or 57.8°C (Bunning et al., 1990).

The control of *Listeria* in food products begins at the lowest level in the processing chain—at the raw product source. The growing environment should be kept free of the opportunity for potential contamination. Waters suspected of contamination by *Listeria*, sewage plant effluent, and sewage sludges should not be used to produce crops that will be eaten without cooking or that will become an ingredient of a product that will be eaten raw. Containers, cartons, boxes, tankers, trucks, or rail cars used to transport the product to the process plant should be frequently cleaned.

At the plant, the raw product itself becomes a source of contamination for the environment. Product flow must be designed to segregate *Listeria*-free food from potentially contaminated environments by control of the product progression through the plant in a manner that separates “clean from dirty,” or processed from raw. Personnel, too, must have their movements within the plant restricted depending on their exposure to potential contamination. There should be no cross-connections between

raw and finished product, whether these cross-connections be humans, equipment, water, air, or the piping arrangement within the plant. If a personnel practice or movement within the plant provides contamination potential, it should be eliminated or modified. If a piping arrangement can, under some circumstances, transport potentially contaminated material from the “dirty” area or process to the finished product area, it should be eliminated, no matter how many valves intervene. Not even the air in the potentially contaminated area should be shared with the finished product area.

Thus, the sanitation regimen of the entire plant must be under constant scrutiny and evaluation. Even with strict sanitation practices, all open product processes should be considered potential contamination points, and careful scrutiny, including microbiological analysis, should be a part of the quality control practice.

Concern cannot stop at the point of packaging; storage and distribution practices must also be evaluated as part of an effective quality control program. The ability of *L. monocytogenes* to withstand harsh environmental conditions such as pH extremes, heat, and freezing requires special considerations when planning for storage and distribution. Surviving microorganisms can grow in products under conditions that would inactivate other microorganisms. Additionally, *L. monocytogenes* is capable of doubling in number each 1.5 days when refrigerated at 4°C (39.2°F). This is of particular concern since more than half the home refrigerators surveyed in a 1999 Audits International study exceeded 39°F (FDA/CFSAN and USDA/FSIS, 2003).

Vibrio species

Joseph M. Madden

The three *Vibrio* species of primary significance in foodborne illnesses are *V. cholerae* (serogroups O1, non-O1, and recently, O139), *V. parahaemolyticus*, and *V. vulnificus*. Another species, *V. mimicus* sp. *novo* (Davis et al., 1981), formerly included in the species *V. cholerae*, also is a recognized pathogen.

Significance as a Pathogen

An estimated 10.1 *Vibrio* infections per million adults who consume raw oysters occur annually in the United

States (FDA/CFSAN, 2001). Between 1981 and 1994, *V. parahaemolyticus* and *V. cholerae* non-O1 caused roughly the same number of illnesses, but gastroenteritis was the common symptom for the former, whereas septicemia occurred more often in cases of the latter (FDA/CFSAN, 2001). *V. vulnificus* was the strain most often responsible for illness, which usually manifested as septicemia (FDA/CFSAN, 2001).

V. cholerae is the bacterium responsible for cholera epidemics and outbreaks. Three groups of *V. cholerae* strains are recognized: serogroups O1, O139, and non-O1. *V. cholerae* O1 is the serogroup traditionally associated with cholera cases, involving severe, watery diarrhea through the action of cholera toxin (Farmer et al., 1985). The serogroup is divided into the Classical and El Tor biotypes (Peterson, 2002). A new serotype of *V. cholerae*, O139, emerged in India in 1992 and spread to neighboring countries. *V. cholerae* O139 also causes cholera, but many secondary infections are asymptomatic (Albert, 1996).

In volunteer feeding studies of serogroup O1, ingestion of 10^6 cells caused illness. The incubation period is typically from six hours to five days (FDA/CFSAN, 2003b). Symptoms of severe cholera include massive diarrhea with large volumes of “rice-water” stool (clear fluid with sloughed-off dead intestinal cells). If left untreated, severe dehydration and death can occur. The massive water loss associated with *V. cholerae* infections has been attributed to the production of toxin by the microorganisms that adhere to and colonize the small intestine of infected individuals. The cholera toxin causes massive fluid loss from cells lining the intestinal tract, and the familiar rice-water stool. Diarrhea can last six to seven days and is usually accompanied by vomiting. Milder forms of diarrhea due to *V. cholerae* O1, lasting one to five days (usually less than three), are more difficult to distinguish from other mild diarrheas (Farmer et al., 1985).

V. cholerae non-O1 can cause a less severe cholera-like illness, but it also causes a much wider spectrum of diseases (Morris et al., 1981), including extraintestinal infections (Hughes et al., 1978). While cholera and gastroenteritis caused by *V. cholerae* O1 is rare in the United States, illnesses caused by the non-O1 serogroup are common and diarrhea usually occurs 48 hr after inges-

tion. *V. cholerae* non-O1 can cause septicemia in susceptible individuals (those with other underlying diseases such as cirrhosis, diabetes, and hemochromatosis, or those on immunosuppressive therapy) via the gastrointestinal tract or wound infections. Septicemia may be accompanied by nausea, but is usually not accompanied by diarrhea. Fever, chills, and nausea may result from the cellulitis occurring with the wound infections.

Only about 3% and 0.2–0.3% of strains of *V. parahaemolyticus* are pathogenic on the West Coast and Gulf Coast, respectively (FDA/CFSAN, 2001). Pathogenic strains of *V. parahaemolyticus* are responsible for outbreaks of acute gastroenteritis, with symptoms of nausea, vomiting, abdominal cramps, low-grade fever, chills, and

Approximately 10–20% of the population consumes raw seafood annually and is at increased risk of infection by *Vibrio* species.

diarrhea (usually watery, sometimes bloody) that begin 12–96 hr after ingestion. The disease is generally mild and self-limiting, lasting from two hours to 10 days, but progression to septicemia can be fatal (Farmer et al., 1985; FDA/CFSAN, 2001).

In Japan, where ingestion of raw seafood is common, *V. parahaemolyticus* is an extremely important diarrheal agent, responsible for 50–70% of enteritis cases (Sakazaki and Balows, 1981). Although outbreaks caused by *V. parahaemolyticus* do occur worldwide, they were uncommon in the United States until four outbreaks involving more than 700 people occurred in 1997–1998. This prompted the FDA to begin a risk assessment of the public health impact of *V. parahaemolyticus* in raw oysters, the food with which they are most often associated (FDA/CFSAN, 2001). A level up to 10,000 viable *V. parahaemolyticus* cells/g was considered acceptable until outbreaks showed that some oysters contained as few as 100 cells/g (FDA/CFSAN, 2001). The FDA

risk assessment estimated that only 15% of illnesses are caused by the consumption of raw oysters containing >10,000 viable *V. parahaemolyticus* cells.

V. vulnificus is primarily associated with serious wound infections and life-threatening primary septicemia (Blake et al., 1979). Primary septicemia is a very serious disease with a 50% fatality rate. Most cases identified also had preexisting liver disease (Blake et al., 1979); however, others have been healthy individuals (Tison and Kelly, 1984). The severe wound infections, which may require amputation or result in death, usually occur after trauma and exposure to marine animals or the marine environment (Blake et al., 1979). The mortality rate associated with wound infections, 7%, is much lower than that associated with septicemia.

V. mimicus is associated with a mild gastrointestinal illness similar to *V. cholera* non-O1, usually occurring after the consumption of raw seafood, particularly oysters (Shandera et al., 1983). The microorganism is probably distributed worldwide in countries situated on an ocean, and its ecology may be similar to that of *V. cholerae* non-O1 (Farmer et al., 1985).

Association with Foods

Approximately 10–20% of the population consumes raw seafood annually and is at increased risk of infection by *Vibrio* species (FDA/CFSAN, 2001). Roughly two-thirds of cases are associated directly or indirectly with seafood; i.e., either by consumption of raw seafood or by consumption of seafood which has been recontaminated after cooking. Oysters are commonly associated with *Vibrio* contamination. In a year-long survey of retail oysters from various locations in North America, 78% of samples from the North Atlantic, Pacific, and Canadian coasts contained less than 0.2 MPN/g of *V. vulnificus*; frequency and levels of *V. parahaemolyticus* were higher. The highest levels of *Vibrio* were in the Gulf Coast (Cook et al., 2002). Some *Vibrio* species may be ubiquitous in the estuarine environment; however, in many cases the presence of *Vibrio* is due to contamination of water by sewage.

Asiatic cholera, caused by *V. cholerae* O1, remains endemic to the Asian continent, and it is suspected that it was reintroduced to the United States in 1973, when the first case of cholera since 1911

was reported. Latin America recently experienced an epidemic affecting nearly one million people and causing more than 9,000 deaths (Guthmann, 1995). There have been occasional outbreaks of cholera in the United States, suggesting that the microorganism has become endemic on the U.S. Gulf Coast, with sporadic cases expected to occasionally occur. Between 1965 and 1991, there were 136 cases of cholera reported to the CDC, of which 93 were acquired in the United States. Fifty-six of the 93 cases were attributed to *V. cholerae* O1. Contaminated crabs and other shellfish harvested near the Gulf Coast were the main source of infection (Weber et al., 1994). Large outbreaks of illness have been eliminated by the establishment of adequate sewage treatment facilities. However, in 1997 more than 90,000 Rwandan refugees became infected with *V. cholerae* O1 (Anonymous, 1998).

V. parahaemolyticus and, because of their physiological similarities, the other vibrios, are found mainly in marine environments throughout the world. However, in a few instances, these vibrios have been isolated from fresh-water or non-marine fish. In these cases, it is assumed that the sodium chloride content of the water was elevated, or the vibrios were present because of pollution of the waters. A correlation between the isolation of these vibrios and water temperatures also exists. Vibrios are isolated more frequently and in higher numbers during the warmer months of the year. Oysters from states along the West Coast and British Columbia contaminated with *V. parahaemolyticus* caused an outbreak in North America in 1997, resulting in 209 cases (FDA/CFSAN, 2003b). In 1998, *V. parahaemolyticus* in oysters and clams harvested from the Long Island Sound caused an outbreak in Connecticut, New Jersey, and New York, and a separate outbreak occurred in the Gulf Coast. The implicated serotype (O3:K6) was recognized as pathogenic in Asia, but had not previously been isolated in the United States. (FDA/CFSAN, 2001).

V. vulnificus is ubiquitous in coastal waters from Florida to Maine. CDC reports that between 1988 and 1995, 302 cases of *V. vulnificus* infections were reported in the Gulf Coast states (CDC, 1996b). An outbreak affecting 16 individuals in Los Angeles was associated with the consumption of raw oysters. The three case fatalities consumed raw

oysters harvested in different places—one in Louisiana and two from two different harvesters in Texas. All three had underlying liver diseases.

Vibrios in general will survive at temperatures below 10°C (50°F), but their reproduction is slowed or arrested at these lower temperatures. An exception is *V. parahaemolyticus*, which dies under refrigeration temperatures of 0–5°C (ICMSF, 1996). At elevated temperatures, the growth of vibrios in contaminated seafood is extremely rapid. Generation times of 12–18 min are common in various seafood held at 30–37°C (86–98.6°F). All of the vibrios are sensitive to drying, yet are able to grow in a wide range of sodium chloride levels, varying from 0.5 to 10% with optimal growth at 2.2% (FDA/CFSAN, 2001).

Control Measures

The distribution of *V. cholerae* and *V. parahaemolyticus* in the environment is better understood than that of the other human pathogenic members of the genus. This is mainly due to the lack of suitable enrichment and primary isolation media for the other vibrios, as well as their recent discovery and association with human illness. These vibrios are, however, thought to be distributed similar to *V. parahaemolyticus* because of their salt tolerance and similar physiological makeup.

Presence in estuarine waters does not correlate with fecal coliform counts; therefore, presence of the bacteria is neither restricted to nor related to poor-quality oyster beds. There is less information concerning the occurrence of *V. mimicus* in the environment and sewage. It has been suggested, however, that this microorganism may also be a normal part of the marine estuarine environment and may also increase in numbers during the warmer months. Vibrio may be distributed throughout different parts of the world through ballast water.

The Interstate Shellfish Sanitation Conference created a Model Ordinance, contained in the Guide for the Control of Molluscan Shellfish, through the National Shellfish Sanitation Program. The document provides the standards for harvesting areas and specifies testing methods for the waters. Because the seafood industry has been required to implement HACCP since 1997, the Model Ordinance also gives details on HACCP for shellfish. The ultimate public health goal is to reduce illness due to *V. vulnificus*

by 60% in 2007 compared to the average level of illness in 1995–1999 in Louisiana, Texas, Florida, and California (NSSP, 2002).

The number of vibrios in estuarine waters tends to increase as the ambient water temperature rises, resulting in their appearance in larger numbers in shellfish and other fishes in these waters during the warmer months of the year (Cook et al., 2002). This may explain the increase in *V. vulnificus* infections in Florida between May and October compared to the rest of the year (CDC, 1993). Special precautions should be taken during these months. The Model Ordinance identifies specific control measures for harvesting areas where two or more cases of *V. vulnificus* have occurred. In those areas, the time between harvesting and refrigeration is halved, from 20 hr to 10 hr, in the summer months. Cooling immediately upon harvesting and maintaining lower temperatures until consumption controls the growth of vibrios in raw seafood. According to the 2001 Risk Assessment conducted by FDA/CFSAN, the annual number of illnesses due to *V. parahaemolyticus* in raw oysters could be reduced from 3,000 to 240, if rapid cooling was employed, or to 15, if oysters were frozen (FDA/CFSAN, 2001). Heating rapidly (5 min, 50°C) kills 4.5–6 logs of all vibrios. The risk assessment estimated that there would be less than 10 cases of *V. parahaemolyticus* infection caused by raw oysters if mild heating was used (FDA/CFSAN, 2001). Adequate cooking and avoidance of recontamination will ensure the safety of seafood.

All of the disease-causing vibrios are naturally occurring in the marine environment and are thus naturally occurring contaminants of seafood, making it impossible to prevent the contamination of these fresh products. The most important control measures to prevent human infections with these microorganisms are hygienic measures designed to prevent their multiplication in these uncooked foods and prevent the recontamination of cooked seafood. Certain individuals (e.g., those with diabetes, those on immunosuppressive drugs, alcoholics, and cirrhotics) should avoid the consumption of any raw seafood at any time of the year. As long as individuals continue to consume raw or undercooked seafood, there will continue to be *Vibrio* infections in the United States.

Staphylococcus aureus

Originally authored by Rosetta L. Newsome. Reviewed by Cynthia M. Stewart.

Given adequate time, temperature, pH, water activity (a_w), and atmosphere for growth, contaminating *S. aureus* may multiply, and many strains may produce enterotoxins when the population exceeds 10^5 cells/g. Ingestion of the thermostable enterotoxins, rather than the bacterium itself, is responsible for foodborne illness.

Significance as a Pathogen

Staphylococcal food intoxication is estimated to cause 185,000 cases of foodborne illness annually (Mead et al., 1999). The true incidence is unknown for several reasons, including poor response by victims to interviews conducted by health officials; misdiagnosis due to symptoms being similar to those of other types of food poisoning (such as the vomiting caused by *Bacillus cereus* emetic toxin); inadequate collection of samples for laboratory analysis; and improper laboratory examination (Ben-nett, 1986).

Unlike other foodborne illnesses, which usually have longer incubation periods, onset of staphylococcal foodborne illness may occur between 30 min and 8 hr following consumption of the toxin-containing food (Bergdoll, 1979). Most illnesses, however, occur within 2–4 hr (Bergdoll, 1979). Staphylococcal enterotoxins (SE) cause severe gastroenteritis (inflammation of the intestinal tract lining). Common symptoms of staphylococcal intoxication include nausea, vomiting, retching, abdominal cramping, sweating, chills, prostration, weak pulse, shock, shallow respiration, and subnormal body temperature. Recovery from this intoxication (which is rarely fatal) usually occurs uneventfully within 24–48 hr.

Growth of staphylococci to a population of a million or more cells per gram of food is considered necessary for sufficient toxin production to elicit symptoms of food poisoning. Several antigenically different protein enterotoxins exist (Tatini et al., 1984). To date, SE A, B, C1, C2, C3, D, E, G, H, I, and J have been identified (Balaban and Rasooly, 2000). Enterotoxin A is the most toxic and the one most commonly involved in staphylococcal food poisoning outbreaks. Data from outbreaks involving

enterotoxin A indicate that less than 1 μ g of toxin can result in illness (Tatini et al., 1984).

Association with Foods

S. aureus is commonly found in the nose and throat (and thus on the hands and fingertips) and on the hair and skin of more than 50% of healthy individuals (Bergdoll, 1979). Any food which requires handling in preparation may therefore easily become contaminated. Infected wounds, lesions, and boils of food handlers may also be sources of contamination, as well as coughing and sneezing by individuals with respiratory infections. *S. aureus* also commonly occurs on the skin and hides of animals, and may thus contaminate foods from these animals as a result of cross-contamination during slaughter.

A variety of foods can support the growth of *S. aureus*. Foods which support growth best include proteinaceous foods, such as meat and meat products, poultry, fish and fish products, milk and dairy products, cream sauces, salads (ham, chicken, potato, etc.), puddings, custards, and cream-filled bakery products. Staphylococci are usually outnumbered by competitive harmless microorganisms in raw foods because they do not compete well with other bacteria, and it is probably for this reason that raw foods are less frequently implicated as the cause of staphylococcal food poisoning. Cooking, however, eliminates the normal competitive bacteria of raw foods; therefore, it is in prepared foods such as meat, potato, and macaroni salads, custards, and cream-filled bakery products that growth of contaminating staphylococci may be permitted.

S. aureus intoxications are often associated with institutions such as schools and prisons where food is often prepared in mass quantities and held until consumption. Lack of sanitation by workers and improper time-temperature combinations can lead to contamination of the product and growth of the microorganism to levels at which toxin is produced. Accordingly, outbreaks can affect a large number of people. An outbreak in 1990 in two schools in Rhode Island resulted from ham prepared at a satellite location that was subsequently handled by an employee who harbored *S. aureus*. The ham was stored at inappropriate temperatures for at least 15 hr and was not adequately reheated (Richards et al., 1993). *S. aureus*,

along with *Salmonella infantis*, was isolated from turkey that caused a foodborne outbreak at a Florida prison in 1990 (Meehan et al., 1992). Methicillin-resistant *S. aureus* (MRSA), commonly associated with hospital infections, caused a foodborne outbreak when a delicatessen employee prepared coleslaw. Tests concluded that the employee carried the outbreak strain of MRSA, which was presumably transferred from a nursing home that the employee frequently visited (Jones et al., 2002).

Although the acidity (low pH) of mayonnaise is inhibitory to the growth of staphylococci, growth may occur in salads (such as egg or chicken), where the otherwise low pH of the mayonnaise is raised or buffered by other salad ingredients. Food systems that contain salt or sugar also provide a favorable environment for growth of *S. aureus*, because growth of more sensitive microorganisms is inhibited, but growth of *S. aureus* is not. Staphylococci can tolerate up to 10–20% salt and 50–60% sucrose. *S. aureus* is also tolerant to nitrite and may therefore multiply in curing solutions or cured meats if they are subjected to conditions favorable for growth.

S. aureus is a facultative anaerobe which grows best under aerobic conditions but is also capable of growth when oxygen is present at reduced concentrations. Some oxygen must be present, however, for toxin production (Bergdoll, 1979; Niskanen, 1977; Tatini, 1973). The microorganism prefers temperatures of 95–98.6°F (35–37°C) but can grow at temperatures as low as 44°F (67°C) or as high as 118°F (47.5°C) (Bergdoll, 1979). While heat processing and normal cooking temperatures are sufficient to kill the bacterial cells, the enterotoxins are heat-stable and are not inactivated by heat processing or cooking. The absence of viable staphylococci, therefore, does not ensure that a food is safe.

Growth may occur at a_w as low as 0.86 under aerobic conditions or at a_w 0.90 under anaerobic conditions. Although staphylococci are fermentative and proteolytic, they do not usually produce off-odors in foods or make them appear spoiled. Presence of the bacteria or their toxins in foods would, therefore, not be detectable by sensory methods.

Control Measures

The ubiquity of the staphylococci in the human and animal environment ne-

cessitates good sanitation in processing operations and food handling, and strict control measures for prevention of bacterial growth and subsequent toxin production. For staphylococcal food poisoning to occur, four things must happen: (1) the food must be contaminated with enterotoxin-producing staphylococci; (2) the food must be capable of supporting the growth of the contaminant; (3) the food must be held at a temperature sufficiently high and for a sufficient period of time to permit sufficient growth to result in the formation of an emetic (vomiting) level of enterotoxin; and (4) the food must be consumed. There is little that can be done about the first two items, because most foods are subject to contamination (for the reasons already given) and are capable of supporting growth. Control of the temperature is the most effective route to control staphylococcal food poisoning. Indeed, the majority of staphylococcal food poisoning outbreaks occur because of inadequate cooling and refrigeration of foods. Adequate heat processing and cooking and proper cooling and refrigeration are therefore important control measures.

Clostridium perfringens **Ronald G. Labbe**

Clostridium perfringens is probably the most extensively studied anaerobic bacterial human pathogen. During the last 90 years, *C. perfringens* has been most closely associated with gas gangrene. The first indication of an association with food poisoning, however, came in the 1940s from Knox and MacDonald in England and McClung in the United States (Hobbs, 1979).

Significance as a Pathogen

Because of the degree of underreporting, estimates of the number of annual cases range from 10,000 to 250,000 in the United States. (FDA/CFSAN, 2003b; Mead et al., 1999). Strains of the microorganism are divided into five toxin types, A to E, based on the production of four lethal extracellular toxins (alpha, beta, epsilon, and iota). Since virtually all food poisoning outbreaks are caused by type A strains, it is not necessary to determine the toxin type in such outbreaks.

Illness due to *C. perfringens* usually occurs 8–22 hr after ingestion of food containing large numbers (10^6 or more)

of vegetative cells. The toxicoinfection is caused by sporulation of the bacterial cells in the intestine, accompanied by production of an intracellular enterotoxin. Sporulation and enterotoxin production may also occur in foods, however (Craven, 1980; Naik and Duncan, 1977). Diarrhea and severe abdominal pain are the usual symptoms. Nausea is less common, and fever and vomiting are unusual. Death is uncommon, usually occurring in debilitated or institutionalized individuals, especially the elderly. Illness generally lasts 24 hr, but may last up to two weeks (FDA/CFSAN, 2003).

Association with Foods

C. perfringens type A can be considered part of the microflora of soil. Virtually all soil samples examined have revealed type A strains at levels of 10^3 – 10^4 /

Since *C. perfringens* will not grow at refrigerated temperatures, the principal cause of virtually all outbreaks is failure to properly refrigerate cooked foods, especially large portions.

g. Types B, C, D, and E are obligate parasites, mostly of domestic animals, and do not persist in soils. The microorganism is also found in the intestinal contents of virtually every animal examined, with wide variation in numbers within and between species. For example, after chilling, carcasses were positive for *C. perfringens* in 13 of 16 broiler flocks. In the affected flocks, 8–68% of individual carcasses harbored *C. perfringens*, with a mean of 30% (Craven et al., 2001). In human infants, adult levels of *C. perfringens* (10^3 – 10^5 /g) are established by six months of age.

Meat and poultry products are by far the most common *C. perfringens* vehicles. This is not surprising, given the incidence of *C. perfringens* in such foods and the microorganism's fastidious nutrient requirements. When 197 comminuted meat samples were tested for the

presence of *C. perfringens* after cooking at 73.9°C, only two samples, both ground pork, had levels above the detection limit of three spores/g (Kalinowski et al., 2003). Nevertheless, in the United States, from 1988 to 1997, meat and poultry items were involved in at least 33% of *C. perfringens* outbreaks; a source was not identified nearly 20% of the time, and multiple vehicles, which may have included meat or poultry were involved in 28% of outbreaks (CDC, 1996a; CDC, 2000b). Fish are not commonly involved in outbreaks, although the body surface and the alimentary canal of most fish of several species harbor the microorganism.

C. perfringens has been found, albeit at a much lower incidence, in virtually all types of processed foods examined. This is important because some of these items, such as spices and herbs, are added to larger volumes of cooked foods. Although processed foods are rarely vehicles for this type of food poisoning, minestrone soup was the vehicle for a 1990 outbreak in Michigan that made 32 people ill (Roach and Sienko, 1992). The soup was prepared two days before consumption and was not promptly cooled or adequately reheated.

Foodservice establishments are the most likely sites for acquiring the illness. This reflects the need for such facilities to cook large amounts of food well in advance of serving. Because of the relatively mild nature of the illness, many outbreaks, especially those involving families, go unreported. The reported incidence is undoubtedly only the tip of the iceberg. The incidence from properly handled commercially prepared food is virtually zero.

Cured meat products are rarely vehicles for outbreaks of *C. perfringens* food poisoning. Two unrelated outbreaks in 1993 were due to improper holding of corned beef. The outbreaks stemmed from the fact that in both instances, the corned beef was prepared in advance in mass in anticipation of the large demand around St. Patrick's Day. The load of *C. perfringens* in corned beef samples exceeded 10^5 CFU/g in each outbreak (CDC, 1994). In general, these products are considered safe when properly handled due to the curing agents themselves, the low initial spore load in such products, the heat treatment administered, and the relative sensitivity to the curing agents of the surviving, and perhaps injured, spore population.

Scientific Status Summary

Control Measures

The problem of *C. perfringens* food-borne illness is one associated with the foodservice industry and consumers. The two most important attributes of *C. perfringens* are its ability to grow rapidly at elevated temperatures and its ability to form spores. Control measures must consider both of these.

Since *C. perfringens* will not grow at refrigerated temperatures, the principal cause of virtually all outbreaks is failure to properly refrigerate cooked foods, especially large portions. An analysis of outbreaks occurring between 1973 and 1987 showed that 97% were due to improper holding temperatures (Bean and Griffin, 1990). Spores on raw meat and poultry can survive cooking and resume vegetative cell growth when the cooling product reaches a suitable temperature. Rapid, uniform cooling is therefore imperative. Prior to 1996, USDA/FSIS prescribed specific time-temperature combinations for the cooking and cooling of RTE meats. In 1999, USDA issued a final rule allowing processors some flexibility in determining the process. With respect to *C. perfringens*, any cooling operation that prevented one log of growth would meet the standard. The process does not need to be validated for uncured meats cooled from 54.4°C (130°F) to 26.7°C (80°F) in less than 1.5 hr, and further cooled to 4.4°C (40°F) in less than 5 hr. (Anonymous, 1999). Cooking also reduces the oxidation/reduction potential and thereby promotes anaerobic conditions, a factor especially important in liquid foods and rolled meats, where the contaminated outside surface is rolled into the middle. Other factors include preparation of food a day or more before serving, inadequate hot-holding, and inadequate reheating of cooked, chilled foods. Cooked foods should be divided into small portions so that refrigeration temperature is reached within two hr. These foods should be reheated to a minimum internal temperature of 74°C (165°F) immediately before serving to destroy vegetative cells. Cooked meat should be kept above 60°C (140°F) or below 4°C (40°F).

It is not possible to prevent carriers of *C. perfringens* from handling food, since all people harbor the microorganism in their intestinal tract. Similarly, the bacterium is present in a wide variety of foods. However, food handlers play a minimal role as a contamination source of *C. perfringens* in meat and

poultry. Thus, preventive measures depend to a large extent on knowledge of proper food preparation and storage techniques, especially temperature control. In the case of *C. perfringens* food poisoning, it is clear that education and supervision of food handlers remain critical control points.

Clostridium botulinum

Merle D. Pierson and N.R. Reddy

The popularity of botulinum neurotoxin (BoNT), also known as “Botox,” to reduce facial wrinkles and the potential to use *C. botulinum* and its neurotoxin as agents of intentional contamination have sparked renewed interest in this pathogen, historically associated with home-canned or prepared products. The etiologic agent of botulism was first isolated from inadequately cured ham in 1896 by E.P.M. van Ermengen. Since *C. botulinum* spores are widely distributed, they may find their way into processed foods through raw food materials or by contamination of foods after processing and cause botulism in humans. Processors and consumers must take preventive measures to eliminate *C. botulinum* or to inhibit growth and toxin production by this microorganism to prevent outbreaks of botulism from occurring. Instances of botulism resulting from improper canning are primarily associated with home canning, not industrial processes. This results in a limited number of cases annually, but does not mean that attention should be diverted from this pathogen.

Significance as a Pathogen

Seven types (A, B, C, D, E, F, and G) of *C. botulinum* are recognized based on the antigenic specificity of toxin. However, all types of *C. botulinum* produce protein neurotoxins with similar effects on an affected host. The types of *C. botulinum* differ in their tolerance to salt and water activity, minimum growth temperature, and heat resistance of spores. All type A strains are proteolytic, and all type E strains are nonproteolytic. Types B and F both contain some proteolytic and nonproteolytic strains. Types A, B, E, and F are involved in human botulism; type C causes botulism in birds, turtles, cattle, sheep, and horses; and type D is associated with forage poisoning of cattle and sheep in Australia and South Africa. No outbreaks of type G have been reported; however,

type G has been isolated in cases of sudden and unexpected death in humans (Sonnabend et al., 1981) and infants (Sonnabend et al., 1985). *C. botulinum* is isolated from a variety of sources, such as soil, marine and lake sediments, feces and carcasses of birds and animals, human autopsy specimens, rotting vegetation, and foods. In addition to these types, *C. baratii* and *C. butyricum*, which produce BoNT, have been isolated from infant botulism cases (Hall et al., 1985; McCroskey et al., 1986; Suen et al., 1988).

C. botulinum produces a BoNT, which is a simple polypeptide that consists of a 100-kDa “heavy” chain joined by a single disulfide bond to a 50-kDa “light” chain. Three-dimensional structure of type A BoNT has been resolved to 3.3Å resolution using data collected from multiple crystals at 4°C (Lacy et al., 1998). BoNTs are toxic to humans and animals; on a weight basis, they are the most lethal substances known, being up to 100,000 times more toxic than sarin (Shapiro et al., 1998). The exact lethal dose of BoNT for humans is not known. However, by extrapolation, Arnon and others (2001) estimated the lethal dose of crystalline type A BoNT for humans from primate data. The lethal dose of crystalline type A BoNT for a 70 kg (154 lb) human would be approximately 0.09–0.15µg via intravenous or intramuscular injection, 0.70–0.90µg via inhalation, and 70µg via oral ingestion (1µg/kg).

Type A BoNT, which causes roughly half the annual cases of foodborne botulism, is more lethal than types B and E, which are equally responsible for the remaining cases (Shapiro et al., 1998). The toxin is a protein that can be inactivated by heat (80°C for 10 min or 85°C for 5 min). The toxin in solution is colorless and odorless and can be absorbed into the blood stream through the respiratory mucous membranes as well as through the wall of the stomach and intestine. Type A BoNT is the first biological toxin licensed for treatment of human diseases, namely for cervical torticollis, strabismus, and blepharospasm associated with dystonia, to reduce facial wrinkles and hemifacial spasm, and for several unlicensed treatments of other human diseases (Arnon et al., 2001; Johnson, 1999; Schantz and Johnson, 1992).

Botulism is currently classified into four categories: (1) classical foodborne

botulism and intoxication caused by the ingestion of preformed BoNT in contaminated food; (2) wound botulism, the rarest form of botulism, which results from the elaboration of BoNT in vivo after growth of *C. botulinum* in an infected wound; (3) infant botulism, in which botulin toxin is elaborated in vivo in the intestinal tract of an infant who has been colonized with *C. botulinum*; and (4) an “undetermined” classification of botulism for those cases involving individuals older than 12 months in which no food or wound source is implicated (Anonymous, 1979; Rhodehamel et al., 1992).

Foodborne botulism results from the consumption of food in which *C. botulinum* has grown and produced toxin. The toxin is absorbed and irreversibly binds to peripheral nerve endings. Signs and symptoms of botulism develop 12–72 hr after consumption of the toxin-containing food. The signs and symptoms include nausea; vomiting; fatigue; dizziness; headache; dryness of skin; mouth; and throat; constipation; paralysis of muscles; double vision; and difficulty breathing. Duration of illness ranges anywhere from 1–10 days or more, depending upon the host resistance, type and amount of toxin ingested, and type of food. Treatment includes administration of botulin antitoxin and appropriate supportive care, particularly respiratory assistance lasting between two to eight weeks (Shapiro et al., 1998). Recovery may take several weeks to months. Death may result; however, the mortality rate is less than 10%.

Infant botulism, which affects infants under 14 months of age, was first recognized in California in 1976 (Midura and Arnon, 1976). This type of botulism is thought to be caused by the ingestion of *C. botulinum* spores that colonize and produce toxin in the intestinal tract of infants (Paton et al., 1982; Wilcke et al., 1980). Honey has been implicated as a possible source of spores. Other non-sterilized foods, as well as nonfood items in the infant's environment, may also be sources of spores. Infant botulism is diagnosed by demonstrating botulin toxins and spores in the infant's stools.

Clinical symptoms of infant botulism start with constipation that occurs after a period of normal development. This is followed by poor feeding, lethargy, weakness, pooled oral secretions, and weak or altered cry. Loss of head

control is striking. Recommended treatment is primarily supportive care. Recent clinical studies have shown that the use of botulism immune globulin (BIG) reduces the time of hospitalization and the need for supportive ventilation and tube feeding (AAP, 2000). Antimicrobial therapy is not recommended (Sakaguchi, 1979).

Due to the rarity of the illness, botulism is often misdiagnosed; however, rapid detection is necessary for the timely administration of antitoxin. Detection of the toxin, either in a suspected food vehicle or the serum or stool of suspected cases, is generally accomplished using an Association of Analytical Communities-approved mouse bioassay. Amplified enzyme-linked immunosorbent assay (ELISA) and amplified

Conditions that favor *C. botulinum* growth and toxin production include a relatively high-moisture, low-salt, low-acid (pH > 4.6) food that is devoid of oxygen and stored without refrigeration.

ELISA/enzyme-linked coagulation assay (ELCA) show promise for more rapid detection of toxin; however, these assays have not yet replaced the mouse bioassay in general use (Ferreira, 2001; Ferreira et al., 2003; Roman et al., 1994).

Association with Foods

Spores of *C. botulinum* are widely distributed in cultivated and forest soils, shore and bottom deposits of streams, lakes, and coastal waters; gills and viscera of crabs and other shellfish; and the intestinal tracts of fish and animals (Eyles, 1986; ICMSE, 1980; Lynt et al., 1975; NFPA/CMI, 1984; Rhodehamel et al., 1992; Sakaguchi, 1979). Although the spores are widespread, only about 22–24 cases of foodborne botulism are reported to the CDC annually (Shapiro et al., 1998; Townes et al., 1996). Type A occurs more frequently in soils of the western regions of the United States, and type B is found more frequently in

the eastern states and in Europe. Type E is most often associated with water environments.

Since fruits and especially vegetables are often in contact with soil, these foods can easily become contaminated with spores of *C. botulinum*. The microorganism has been isolated from fresh and processed meats, but the incidence is generally low (Hauschild and Hilsheimer, 1980; Rhodehamel et al., 1992).

C. botulinum spores have been detected in honey and corn syrup (Lynt et al., 1982). *C. botulinum* is a natural contaminant of fish. The intestinal tract of fish and its contents have been reported to be the main reservoirs (Huss et al., 1974). Since many processes do not include steps that are lethal to spores of *C. botulinum*, the microorganism may be found occasionally in some finished products, such as smoked fish.

Proteolytic types A, B, and F strains produce heat-resistant spores that are a major concern in the processing of low-acid canned foods. The nonproteolytic types B, E, and F strains produce spores of low heat resistance and cause problems primarily in pasteurized or unheated foods (Sperber, 1982). Proteinaceous foods, such as meats, and nonproteinaceous foods, such as vegetables, can provide sufficient nutrients for growth and toxin production by *C. botulinum*. The proteolytic types digest proteins in foods and produce foul odors, which may warn consumers. However, there have been outbreaks where only a small amount of food having an off-odor was consumed or food without evidence of spoilage was consumed. The latter is especially true for the nonproteolytic strains (Lynt et al., 1975).

The proteolytic and nonproteolytic types of *C. botulinum* differ in their tolerance to salt, water activity, minimum growth temperature, and spore heat resistance. The optimum temperature for growth and toxin production by the proteolytic types is about 35°C (95°F), with very slow growth at both 12.5°C (55°F) and 50°C (122°F); however, at the latter temperature, toxin may be slowly inactivated (Bonventre and Kempe, 1959). The nonproteolytic types can grow between 3.3°C (38°F) and 45°C (113°F), with an optimum for growth and toxin production at about 30°C (86°F). Refrigeration above 3.3°C may not be a complete safeguard against botulism for foods containing nonproteolytic strains (Eklund et al., 1967; Patel et al., 1978).

Scientific Status Summary

Almost every type of food product (dairy products, vegetables, jarred peanuts, fishery products, meat products [beef, pork, and poultry], and condiments [chili sauce, chili peppers, tomato relish, and salad dressing]) has been implicated in foodborne botulism outbreaks (Arnon et al., 2001; Aureli et al., 2000; Chou et al., 1988; Kalluri et al., 2003; Peck, 2002; Rhodehamel et al., 1992; Weber et al., 1993). The largest U.S. outbreak since 1978 occurred in 1994 as the result of improper storage of baked potatoes later used to make dip. Of the 30 reported cases, four required mechanical ventilation (Angulo et al., 1998). Cheese and other dairy products were implicated in less than 1% of reported cases of *C. botulinum* intoxication since 1899 (Townes et al., 1996). Improper handling and storage of commercially canned cheese was responsible for a restaurant outbreak in 1993 affecting eight people and resulting in one death (Townes et al., 1996). In 1989, toxin production in garlic-in-oil caused three cases of botulism. Since this was not the first time this type of product, which had a pH >4.6, was implicated, FDA began requiring that products of this nature be acidified or contain antimicrobial agents (Morse et al., 1990). FDA issued guidance for labeling of foods requiring refrigeration following botulism outbreaks caused by black bean dip and clam chowder. Illness could have been prevented had the items, which were not commercially sterile, been properly refrigerated by the consumer. Although the products were displayed in the refrigerated section of the grocery store, FDA felt that because of the packaging consumers did not follow the directions for refrigeration. Temperature abuse allowed the growth of *C. botulinum* to toxin-producing levels. To prevent future outbreaks, FDA recommended that foods that may present a safety risk if not refrigerated bear the label, "IMPORTANT: Must Be Kept Refrigerated" (FDA, 1997).

Control Measures

Conditions that favor *C. botulinum* growth and toxin production include a relatively high-moisture, low-salt, low-acid (pH > 4.6) food that is devoid of oxygen and stored without refrigeration (above 38°F or 3.3°C). The food industry uses a variety of physical and chemical treatments to either destroy *C. botu-*

linum spores or control growth and subsequent toxin production. Reviews of specific treatments and hurdle concepts used for control of *C. botulinum* in a variety of food products have been published (Hauschild, 1989; Rhodehamel et al., 1992).

Typical methods used to prevent botulism include reduction of vegetative cell and/or spore contamination in the food by heat treatment (e.g., pressure cooking for canning) to obtain "commercial sterility" and use of lower heat treatments (pasteurization) in combination with other control measures. Nitrite and salt (in a brine solution) are control measures used in addition to heat treatment for low-acid canned meats (e.g., canned ham). In addition to these controls, refrigeration is an important control for perishable vacuum-packaged meats. Acidification is used for other food products such as pickles, mayonnaise, and canned fruit products. Reduction of moisture level (drying) below an a_w of 0.93 is employed in certain foods. Frozen storage is yet another significant factor in controlling pathogen growth and subsequent toxin formation.

The excellent safety record of cured meats relative to botulism outbreaks has been largely attributed to the use of nitrite as a curing ingredient. Many studies have been published on the efficacy of sodium nitrite in inhibiting *C. botulinum* growth and toxin production in perishable cured meats such as wieners, bacon, canned ham, luncheon meat, and canned comminuted meat (Rhodehamel et al., 1992). In perishable cured meats, safety cannot be totally attributed to nitrite alone, but a variety of factors and their interaction with nitrite provide protection against botulinal growth and toxin production. Factors such as heat treatment, acidity (pH), salt, and bacterial spore level influence the effect of nitrite on *C. botulinum* growth and toxin production. Other curing adjuncts, such as ascorbic acid and sodium erythorbate, may also influence the efficacy of nitrite.

The greater incidence of botulism due to improper home processing and storage of foods, particularly home-canned foods, compared to commercially processed foods reflects the food industry's greater awareness and control of the key factors in inhibition of *C. botulinum* growth and toxin production. Consumers must recognize that certain

control measures, such as proper refrigeration and frozen storage, must be maintained from purchase until consumption. If a commercial product is mishandled, control measures are compromised and botulism may result.

Bacillus cereus

Michael P. Doyle

Bacillus cereus has been a recognized cause of foodborne illness for almost 50 years. Two types of illnesses, the emetic and diarrheal responses, are caused by two distinct enterotoxins produced by this microorganism (Melling et al., 1976).

Significance as a Pathogen

An estimated 27,000 cases of foodborne illness due to *B. cereus* occur annually in the United States (Mead et al., 1999). A large molecular weight protein causes the diarrheal response, whereas cereulide, a small peptide stable for 20 min at 121°C, causes the emetic response (Granum, 1997). The diarrheal response, which closely mimics symptoms of the illness caused by *C. perfringens*, generally produces mild symptoms that usually develop between 6–15 hr after ingestion. The emetic (or vomiting) response occurs within a few (one to six) hr after ingestion, closely mimicking symptoms produced by staphylococcal enterotoxin. Symptoms of the diarrheal syndrome include diarrhea, abdominal cramps, and tenesmus, whereas nausea and vomiting are the principal symptoms of the emetic syndrome. Recovery is usually complete within 24 hr after onset, and further complications do not occur. The diarrheal syndrome results following ingestion of large numbers of the microorganism, whereas the emetic syndrome is likely to result from ingestion of preformed toxin in the food.

Association with Foods

B. cereus is common in soil and on vegetation and can be readily isolated from a variety of foods, including dairy products, meats, spices and dried products, and cereals (especially rice). In view of its wide distribution in the environment, *B. cereus* is ingested from time to time and inevitably is part of the transitory human intestinal microflora. Studies have revealed that low numbers of *B. cereus* are present in the intestinal

tract of more than 10% of the healthy adult population (Ghosh, 1978; Shinagawa et al., 1980).

Foods that have been implicated as vehicles in outbreaks of *B. cereus* diarrheal-type food poisoning include cereal dishes that contain corn and corn starch, mashed potatoes, vegetables, meat products, puddings, soups, and sauces. *B. cereus* emetic-type food poisoning is most frequently associated with fried or boiled rice dishes (Johnson, 1984). Chicken fried rice was implicated in a 1993 outbreak in Virginia that affected 14 people (FDA/CFSAN, 2003b). Other starchy foods such as macaroni and cheese also have been incriminated as vehicles of the emetic syndrome.

B. cereus is a spore-forming microorganism whose spores will generally survive cooking. Spores of the microorganism normally occur on many foods from harvest through primary processing. The microorganism is not considered a hazard at the very low levels typically present in food. However, problems develop when food (especially cooked foods which are devoid of most heat-sensitive microbial competitors) is held at 10–55°C (50–131°F) for a long period of time. Under these conditions, the microorganism grows to large numbers, releasing toxin during growth in the food and in the intestinal tract following ingestion. Although ingestion of more than 10⁵ cells/g is required to produce illness (Hobbs and Gilbert, 1974), ingesting large numbers of *B. cereus* does not always produce illness (Dack et al., 1954). To date, all outbreaks of both types of *B. cereus* illness have resulted because of improper holding temperatures or slow cooling of large amounts of food. Because these illnesses are intoxications, they are not infections and are not transmitted. It does not appear that processing of raw agricultural commodities contributes to the incidence of *B. cereus* illness.

Control Measures

Measures to reduce or eliminate *B. cereus* food poisoning are well established. Most disease incidents result from time-temperature abuse of food in food-handling establishments. This can be prevented by holding foods at greater than 60°C (140°F) until served, or rapidly cooling foods to below 10°C (50°F) for storage. Refrigerating foods in small

quantities will facilitate rapid cooling. Ideally, foods should be cooled to below 15°C (59°F) within two to three hr after cooking. Regarding preparation of rice and fried rice, the following recommendations have been made: (1) prepare quantities of rice as needed; (2) keep prepared rice hot (55–63°C, 131–146°F); (3) cool cooked rice quickly; and (4) re-heat cooked rice thoroughly before serving (Bryan et al., 1981; Gilbert et al., 1974; Moreland, 1976; PHLS, 1976; Sly and Ross, 1982).

B. cereus spores in foods can be destroyed by retorting (pressure cooking) or applying an equivalent thermal process or by irradiation. However, such treatments may be too severe and, hence, unacceptable for many foods.

Enterobacter sakazakii

Jennifer C. McEntire and Francis F. Busta. Reviewed by R. Bruce Tompkin

Advances in medicine have vastly improved the mortality rate for infants. Unfortunately, this population is particularly susceptible to often-fatal infection by *Enterobacter sakazakii*, introduced through reconstituted powdered infant formula.

Significance as a Pathogen

As of 1989, 20 cases of neonatal infection by *E. sakazakii* were reported worldwide (Biering et al., 1989). By 2003, 48 neonatal cases were recognized (FDA/CFSAN, 2003b). Within the last several years, awareness of the problem associated with this pathogen has increased; as a result, research and reports of *E. sakazakii* infection in infants have also risen.

In 1980, yellow-pigmented *Enterobacter cloacae* was recognized as its own species—*E. sakazakii*. Although infection by this microorganism has been reported since the 1960s, the pathogen was not recognized as a cause of food-borne illness because the ecology of the microorganism was unknown.

Infection by *E. sakazakii* is an extremely rare event. Six of the 58 reported cases of *E. sakazakii* infection worldwide involved individuals more than four years of age, and the median age was 74 (FDA/CFSAN, 2003b). The vast majority (83%) of cases have been reported in infants less than one year of age, where the fatality rate ranged from

30% to 80% despite antibiotic treatment (Muytjens et al., 1983). There have been conflicting reports regarding low birth weight as a risk factor for infection (FDA/CFSAN, 2002; Muytjens et al., 1983; van Acker, 2001); however, the Contaminants and Natural Toxicants Subcommittee of the FDA/CFSAN's Food Advisory Committee reviewed risk factors for *E. sakazakii* infections and concluded that infants born at less than 36 weeks gestational age were at risk until six weeks post-term. Other risk factors identified were hospitalization in level-two or level-three neonatal intensive care units and immunocompromised health status (FDA/CFSAN, 2003a).

Illness symptoms normally appear a few days after birth, and the health of the infant rapidly deteriorates (Muytjens et al., 1983). Infection may result in meningitis (58%), necrotizing enterocolitis (29%), or sepsis (17%). In one outbreak in which 11 infants were positive for *E. sakazakii*, one developed meningitis and four others had clinical signs of severe sepsis, although the microorganism could not be isolated from the blood (Arseni et al., 1987). Bacteremia is often, but not necessarily, confirmed (Muytjens et al., 1983).

The severe consequences of infection in some cases may be linked to the production of enterotoxin by *E. sakazakii*. More than 20% of the 18 tested strains produced enterotoxin. High levels (10⁸ cfu) of each strain were lethal to mice when administered by intraperitoneal injection. Peroral administration of the same levels were only lethal for two strains (Pagotto et al., 2003).

When infection does not result in death, the affected infant may have permanent neurological or developmental deficiencies. In one case, mortality was averted by months of antibiotic treatment, but the patient was mentally retarded and paralyzed by age two (Biering et al., 1989). In another study of eight infected infants, the two survivors were both retarded, suffered from hydrocephalus, and eventually died (Muytjens et al., 1983). However, full recovery is also possible (Simmons et al., 1989).

Infants may be colonized with *E. sakazakii* without developing symptoms (Arseni et al., 1987; FDA/CFSAN, 2002). Although colonization and fecal carriage may last 8–18 weeks, secondary

Scientific Status Summary

transfer is not known to occur (Arseni et al., 1987; Block et al., 2002).

Association with foods

Although powdered infant formula was postulated as the vehicle for *E. sakazakii* infection as early as 1983 (Muytjens et al., 1983), the first conclusive evidence linking the infectious microorganism with the product was in 1989 (Biering et al., 1989). After several cases of infant infection in Iceland, tests of the powdered milk showed that the microorganism could be isolated from all lot numbers examined. However, neither every individual sample nor every package from a lot showed *E. sakazakii* contamination after enrichment, indicating that the microorganism was present at low levels. Plasmid analysis and antibiograms of 22 out of 23 strains isolated from the formula were identical to the strains that caused illness (Biering et al., 1989). These techniques, as well as chromosomal restriction endonuclease analysis, ribotyping, arbitrarily primed PCR, and multilocus enzyme electrophoresis, have been used to conclusively link outbreak strains with strains isolated from formula in several other outbreaks (Clark et al., 1990; Simmons et al., 1989; van Acker et al., 2001).

After a fatal case of *E. sakazakii* infection was confirmed in a premature infant in Tennessee, 49 other infants in the hospital were screened for infection or colonization over a 10-day period. The microorganism colonized in seven asymptomatic infants and caused either confirmed or suspected infection in three others. Pulsed-field gel electrophoresis confirmed that the strain of *E. sakazakii* isolated from the cerebral spinal fluid of an infected infant was identical to the strain cultured from opened and unopened packages of infant formula (FDA/CFSAN, 2002). As a result, the implicated lot was recalled. A separate, unrelated recall of powdered infant formula produced by a different manufacturer was conducted in 2003, again due to the presence of *E. sakazakii*.

A 2002 FDA field survey collected 22 samples of finished infant formula from eight different plants. Five samples (22.7%) were positive for *E. sakazakii* at the lowest detectable level, 0.36 MPN/100 g. The positive samples included four out of 14 formulas for full-term infants and one of four made specifically for pre-term infants. The study showed that the percent of positive samples was

similar regardless of whether the formula was manufactured through wet-mixing, spray drying, or dry blending, or if the formula was made with milk or soy (FDA/CFSAN, 2004). A survey of powdered infant formula from 35 countries showed that more than 14% of samples were positive for *E. sakazakii*. This included two of 12 samples from powdered infant formula purchased in the United States. The levels of *E. sakazakii* were low—0.36 and 0.92 cfu/100 g (Muytjens et al., 1988). Levels of the pathogen were less than 1 cfu/100 g in 17 of 20 positive samples, and all tested

Although *E. sakazakii* contamination of powdered infant formula occurs at very low levels, generally less than 1 cfu/100 g, some infants with the associated risk factors are susceptible to infection.

samples complied with Codex Alimentarius recommendations for coliform counts. The Codex standard requires four of five samples to have no positive tubes for coliforms in a three-tube MPN; the fifth sample may contain up to 20 cfu/g (FAO, 1994). The batch of powdered infant formula implicated in a Belgian outbreak also met the specifications of Codex Alimentarius, but not the more stringent Belgian law which requires all samples to have less than 1 cfu/g. Levels of *E. sakazakii* were so low that the microorganism was not initially detected in the dry powder, and its continued use resulted in another case (van Acker et al., 2001).

Control measures

Although *E. sakazakii* contamination of powdered infant formula occurs at very low levels, generally less than 1 cfu/100 g, some infants with the associated risk factors are susceptible to infection. The current control measure used by hospitals to prevent infection is the use

of commercially sterile liquid formula in lieu of powdered infant formula for at-risk infants. The FDA/CFSAN Advisory Committee subcommittee (2003a) charged with determining if there was a link between *E. sakazakii* infection and powdered infant formula reiterated this recommendation.

To decrease the risk of infant infection by *E. sakazakii*, FDA prepared guidance for the preparation and use of powdered infant formula in neonatal units (FDA/CFSAN, 2002). Time and temperature control are key. Initially, guidance proposed using boiling water to reconstitute the powder and destroy the microorganism, but essential nutrients could potentially be destroyed as well. The thermotolerance of the microorganism seems to be strain-dependent with the more tolerant strains having $D_{58^{\circ}\text{C}}$ values up to 600 sec (Edelson-Mammel and Buchanan, 2004). While pasteurization should be sufficient to kill the pathogen, the microorganism may be more heat resistant than other *Enterobacteriaceae* (Breeuwer et al., 2003; Nazarowec-White and Farber, 1997). Edelson-Mammel and Buchanan (2004) showed that preparing formula with hot, but not boiling, water (i.e., 70°C) is sufficient to reduce *E. sakazakii* by more than 3.9 logs within about 15 sec. Although changes in preparation and storage of formula may help prevent infections, guidance and recommendations do not address reducing contamination at the production level.

The survival of *E. sakazakii* in powdered infant formula may be partially due to the ability of the microorganism to survive desiccation. Breeuwer et al. (2003) speculated that this is related to trehalose accumulation in stationary phase cells. Although the microorganism presumably does not survive pasteurization, it may be able to survive the conditions of powdered milk if introduced during or after drying. This is consistent with Muytjens et al. (1988), who speculated that the low levels of the pathogen indicate that *E. sakazakii* is introduced through post-process contamination.

An understanding of the environmental reservoir(s) would facilitate control of the pathogen. Until recently, this was unknown. Insects recently have been suggested as carriers of *E. sakazakii*. For example, Mexican fruit flies were shown to harbor the microorganism (Kuzina et al., 2001). Hamilton et al.

(2003) reported the isolation of *E. sakazakii* from the larvae of *Stomoxys calcitrans*, commonly known as the stable fly. Rats also have been identified as a source of the pathogen (Gakuya et al., 2001). These reports stress the importance of appropriate pest control as a means to reduce the presence of *E. sakazakii* in production facilities. The presence of the microorganism in five of 16 homes and eight of nine manufacturing facilities producing a variety of dried foods suggests this opportunistic pathogen is more ubiquitous than had been previously assumed, making control more difficult (Kandhai et al., 2004).

The FDA/CFSAN Advisory Committee subcommittee (2003a) encouraged microbiological sampling and testing for *E. sakazakii* in powdered infant formula. Biochemical assays can be used to identify *E. sakazakii* and distinguish it from related *Enterobacteriaceae*, and the FDA is developing a real time PCR-based system for rapid detection (Seo et al., 2003). Dupont Qualicon has already included *E. sakazakii* in its BAX® PCR-based detection system (Dupont, 2003). The committee also recommended that manufacturers formulate products to maximize microbiological safety, and design and operate plants under hygienic conditions. Currently, FDA does not have GMPs for powdered infant formula. The committee noted, however, that these interventions can not completely eliminate the risk of *E. sakazakii* infection to infants fed reconstituted powdered formula.

The risk reduction strategies suggested by a joint FAO/WHO committee made similar recommendations, and conducted a risk assessment to show the effects of possible interventions (WHO, 2004). The group recommended that Codex develop microbiological specifications for *E. sakazakii* in powdered infant formula. An IFT authoritative report on performance standards outlines the questions that would need to be answered in order to develop such specifications (IFT, 2004).

Conclusion

When the first edition of this Scientific Status Summary was published in 1988, *E. coli* O157:H7 and *L. monocytogenes* were emerging pathogens that were not yet "household names." *Campylobacteriosis* was just being recognized as the cause of illnesses previously identified as salmonellosis, and al-

though one report isolated *E. sakazakii* from powdered milk, the cause of infection in neonates was largely unknown. Strides have since been made to improve food safety, and fortunately, some of the pathogens discussed are currently recognized as minimal contributors to foodborne illness. The emergence of new pathogens continues, not unexpectedly, as the cause of foodborne illness is unknown in 81% of cases (Mead et al., 1999). This Scientific Status Summary has focused on the bacteria of significance in foodborne disease. The pathogenic (disease-causing) bacteria discussed in this summary cause most of the known bacterial foodborne illness in North America. Certain characteristics of these bacteria have been well known for decades, while others have appeared more recently in somewhat unexpected situations.

The principal control measures for prevention of foodborne diseases continue to be use of adequate time-temperature heat treatments or cooking procedures; avoidance of cross-contamination of cooked or RTE foods by utensils, equipment, or cutting surfaces that are not properly cleaned and disinfected after contact with fresh, uncooked raw foods; avoidance of consumption of undercooked or contaminated raw foods; and avoidance of contamination of raw foods during handling by infected food handlers.

The majority of serious foodborne disease outbreaks occur as the result of improper attention to detail in foodservice establishments and in the home. The need for education of all food handlers, including consumers, is readily apparent.

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