Review

Salmonellosis Outbreaks in the United States Due to Fresh Produce: Sources and Potential Intervention Measures

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Abstract

Foodborne *Salmonella* spp. is a leading cause of foodborne illness in the United States each year. Traditionally, most cases of salmonellosis were thought to originate from meat and poultry products. However, an increasing number of salmonellosis outbreaks are occurring as a result of contaminated produce. Several produce items specifically have been identified in outbreaks, and the ability of *Salmonella* to attach or internalize into vegetables and fruits may be factors that make these produce items more likely to be sources of *Salmonella*. In addition, environmental factors including contaminated water sources used to irrigate and wash produce crops have been implicated in a large number of outbreaks. *Salmonella* is carried by both domesticated and wild animals and can contaminate freshwater by direct or indirect contact. In some cases, direct contact of produce or seeds with contaminated manure or animal wastes can lead to contaminated crops. This review examines outbreaks of *Salmonella* due to contaminated produce, the potential sources of *Salmonella*, and possible control measures to prevent contamination of produce.

Introduction

RECENT ADVANCES IN PROCESSING TECHNIQUES, preservation, and packaging have enabled the fresh fruit and vegetable industry to consistently supply consumers with a wide array of products year round in much greater quantities. Produce consumption in the United States has risen in recent years due to the increase of public health interests and diet trends. The sales of salad mixes alone increased from \$167.5 million in 1991 to \$507 million in 1994 (E. Garrett, International Fresh Cut Produce Association, Arlington, VA, personal communication, 1996). The increased consumption nearly doubled production values of lettuce from \$1.3 billion dollars in 1999 to \$2 billion in 2004 (USDA-ERS, 2005). It is generally perceived that consumption of raw fresh produce products is safe and that most foodborne disease outbreaks are caused by foods primarily of animal origin (Sivapalasingam *et al.*, 2004). However, the potential for foodborne pathogen contamination is apparent in many aspects of vegetable production practices. Accordingly, fresh fruit and vegetables have been implicated

in a myriad of foodborne outbreaks and as sources of foodborne pathogens (Doan and Davidson, 2000; Sewell and Farber, 2001; Castillo and Rodríguez-García, 2004; Mandrell and Brandal, 2004; Sivapalasingam *et al.*, 2004).

Fresh fruits and vegetables may be contaminated with either spoilage or pathogenic microorganisms through cultivation practices, handling, and processing (Tournas, 2005). Because the addition of preservation methods is often absent in vegetable processing, there is a potentially greater risk for microbial populations to contaminate and deteriorate the product (Thomas and O'Beirne, 2000). Recent documented foodborne disease outbreaks associated with fresh produce have been on the rise. Bean and Griffin (1990) reported that fruits and vegetables were the cause of 2% of the foodborne disease outbreaks in the United States between 1973 and 1987. By the 1990s, this had risen to 6% of all reported foodborne outbreaks with over 16,000 cases of illness identified from fresh produce and produce dishes in the United States (Sivapalasingam *et al.*, 2004; Mandrell and Brandal, 2004). The most recent data indicate that 13% of outbreaks in the United

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States may be attributed to produce contaminated with foodborne pathogens (Doyle and Erickson, 2007).

As demand for vegetables and other crops continues to rise, the need for freshwater sources to provide irrigation also increases. This can be especially problematic for freshwater sources that come in contact with regions where there are large confined animal operations or large numbers of grazing animals. In the western United States animal grazing is substantial enough to impact water quality (Larsen *et al.*, 1994). In addition, the application of animal wastes to agricultural soils can increase levels of foodborne pathogens originating from the manure of animal carriers (Crane *et al.*, 1983; Edwards and Daniels, 1992; Solomon *et al.*, 2002; Hutchison *et al.*, 2004). In areas that are more densely populated or are closer to large municipalities, raw sewage waste also can be a source of human pathogens (Ait Melloul *et al.*, 2001).

The globalization and importation of the food supply has resulted in unique food safety challenges. The cooperation of international agencies, formation of worldwide regulations, and enhanced surveillance systems are indispensable for a safe food supply (Kaferstein, 2003). The United States Department of Agriculture-Economic Research Service (USDA-ERS, 2008) estimates 13% of vegetables and 32% of fruits and nuts consumed in the United States in 2007 were imported, up from 16.4% total fruits and vegetables in 1996. The burden of inspecting these imported food items is placed on regulatory agencies that only inspect about 1% of imported food (Buzby *et al.*, 2008). Because greater volumes of fresh fruits and vegetables are being imported along with greater volumes being consumed, it is expected that the number of foodborne illnesses due to produce will increase (Harris *et al.*, 2003).

Salmonella is a leading cause of foodborne illness in the United States (CDC, 2008a). The USDA-ERS estimated 1.4 million cases of salmonellosis occurred in 2007 costing 2.5 billion dollars in terms of lost productivity and medical costs (USDA-ERS, 2007). *Salmonella* has been shown to survive or grow in a wide variety of produce. This review will focus on produce that has been implicated in foodborne outbreaks in the United States. Although outbreaks can also be attributed to multiple sources including food preparers and the food preparation environment, contamination of produce early in the production chain such as the field and processing environment will be the focus of this review. The environmental aquatic sources of *Salmonella* contamination of produce also are examined. Finally, control measures for limiting *Salmonella* contamination in produce are discussed.

Produce Outbreaks Due to *Salmonella*

Salmonellae are commonly found in the gastrointestinal tract of numerous animals such as birds, reptiles, farm animals, and humans. Most cases of salmonellosis were previously thought to be attributed to consuming contaminated poultry and poultry products (Tauxe *et al.*, 1997). However, an increasing number of produce-associated foodborne outbreaks in the United States associated with bacterial contamination are primarily from *Salmonella* (Tauxe *et al.*, 1997; Harris *et al.*, 2003). In fact, from 2002 to 2003, 31 produce-associated outbreaks of *Salmonella* were reported while only 29 outbreaks were poultry related (CDC, 2008a). A wide variety of produce has been shown to be a source of *Salmonella*

outbreaks including tomatoes, sprouts, watermelon, cantaloupe, lettuce, and mangoes (Tauxe *et al.*, 1997). Outbreak investigations of *Salmonella* infection implicated watermelons as early as 1950 (Harris *et al.*, 2003).

Produce-linked outbreaks of *Salmonella* are summarized in Table 1. Typically in larger outbreaks, the source of *Salmonella* that contaminated the produce is eventually identified. However, trace-back investigations can be hindered due to the complexity of the food supply. Information such as brand name, date of purchase, Universal Product Code (UPC) code, and lot numbers are important for pinpointing a contaminated food item and may not be available. Furthermore, trace-back investigations rely on consumers that have become ill for information and may only provide limited information because 1) only a limited number of cases are available; 2) people that are interviewed typically have a limited recall about the foods that were eaten; and/or 3) the food source of the outbreak is no longer available for testing. As demonstrated by the recent 2008 outbreak of salmonellosis due to Serrano peppers, an investigation can be hindered if the right questions are not asked; in the 2008 outbreak, initial questionnaires by investigating agencies did not specifically ask if peppers were recently eaten (CDC, 2008b). Furthermore, it is estimated that only 1 case of salmonellosis out of 38 may be reported to the public health authorities and this also limits outbreak investigations (Voetsch *et al.*, 2004). Outbreaks due to specific produce items are discussed in the following sections. In most cases, details of the larger outbreaks were available and are given.

Melon

Melons have been associated with multiple salmonellosis outbreaks. Between 1989 and 1991, two consecutive outbreaks that sickened more than 400 people occurred due to contaminated cantaloupe originating from Mexico and Texas (CDC, 1991). Several past outbreaks have also been associated with watermelon but comparatively fewer cases were reported (Gayler *et al.*, 1955; CDC, 1979). If the outside of the melon is contaminated, cutting the melon can transfer *Salmonella* to the edible portions (Gayler *et al.*, 1955; Ukuku and Sapers, 2007). Since melons are rich in sugars and nutrients and have a nearly neutral pH (Gagliardi *et al.*, 2003), it is not surprising that when contaminated melon pieces are not stored properly (i.e., room temperature) *Salmonella* populations can increase (Ukuku and Sapers, 2007).

The contamination of melons can be from multiple sources including contaminated manure, irrigation water, fertilizers, wildlife, wash water, processing equipment, and/or packaging (Kaneko *et al.*, 1999). However, a survey of farm and processing facilities found that a majority of *Salmonella* contamination resulted from the rind being inoculated during immersion in contaminated wash water in post-harvest facilities (Gagliardi *et al.*, 2003). Water in the wash tank or coolers that becomes contaminated can inoculate large numbers of melons subsequently washed in the same water. Cantaloupes, in particular, are difficult to clean once they are inoculated because the surface of the melon has lenticles (netting) that provide areas for attachment and protection from sanitizers (Lester, 1988; Gagliardi *et al.*, 2003). Vigorous scrubbing with multiple detergents and disinfectants is necessary to remove contamination and may not remove all of the pathogens (Barak *et al.*, 2003; Parnella *et al.*, 2005).

Therefore, preventing the initial contamination of melons in the wash tank is considered a critical control point.

The voluntary Melon Quality Control Program was initiated by the produce industry in 1992 because of the increasing number of *Salmonella*-contaminated melon products (unpublished discussion, Melon Quality Program meeting, Dallas, TX, October 14, 1991; Mohle-Boetani *et al.*, 1999). Recommendations coming out of this program include the use of hyperchlorinated water in all processing steps of fruit and vegetable production to help reduce bacterial contamination (Tauxe *et al.*, 1997). However, this program is voluntary for U.S. producers and imported melons may not follow these guidelines. As a result, outbreaks of salmonellosis from imported melons continued after the program implementation as demonstrated by the 2008 outbreak due to cantaloupe imported from Honduras (FDA, 2008a).

Careful monitoring of chlorine levels in wash water is critical because chlorine levels can be rapidly neutralized by organic materials washed from the melons. In addition, chlorine may produce off-flavors and undesirable appearance of foods, and may be harmful to the environment. Alternatives to chlorination have been studied. Bacteriophage treatment was reported to effectively reduce *Salmonella* populations on cut melon by 3.5 log₁₀ (Leverentz *et al.*, 2001). However, like antibiotics, *Salmonella* can become resistant to bacteriophages, which eventually renders this treatment ineffective (Santander and Robeson, 2007). Hydrogen peroxide application has also been demonstrated to be effective, but only if treated within 24 hours of contamination (Ukuku and Fett, 2002). Similarly, commercial sanitizers were only effective if used within 1 hour of inoculating melons (Ukuku and Sapers, 2007).

The need for effective treatments that can disinfect contaminated melons remains. The genetic basis for *Salmonella* attachment to plants has been discovered and this information could be used to develop effective treatments. It has been demonstrated that *Salmonella* regulates production of cellulose using the *bcsA* gene and the O-antigen capsule gene *yihO* in order to attach to and colonize plants (Barak *et al.*, 2007). A key regulator of the O-antigen capsule and cellulose production is the positive regulator AgfD (Zogaj *et al.*, 2001; Barak *et al.*, 2005). In addition to regulation of these genes, AgfD is also suggested to be a key regulator for survival of *Salmonella* outside the host (Barak *et al.*, 2007). Thin aggregative fimbriae may also be used for attachment and genes required for expression of fimbriae include *rpoS* and *agfD* (Romling *et al.*, 2005). Importantly, these genes are also necessary for virulence expression during human infection (Barak *et al.*, 2005).

Using this information, potential treatment options could be explored by monitoring *Salmonella* genes after exposure to treatments to determine any effect on attachment and virulence. For example, organic acids have been explored as antimicrobial treatments and the organic acids butyrate, propionate, and acetate all have biocidal effects against *Salmonella* (Ricke, 2003; Van Immerseel *et al.*, 2006). Butyrate and propionate decreased expression of *hilA* (virulence gene regulator), *invF* (cell invasion factor), and *sipC* (cell invasion protein), but acetate led to an increase of *hilA* and *invF* (Durant *et al.*, 2000; Lawhon *et al.*, 2002). Therefore, acetate may not be a good choice as an antimicrobial due to increases in virulence expression. Furthermore, any *Salmonella* surviving exposure to an organic acid may be more likely to

survive further acid treatment due to the induction of an acid tolerance response and produce cross-protection to other antimicrobials (Kwon and Ricke, 1998; Kwon *et al.*, 2000; Ricke, 2003).

Sprouts

In 1999, the U.S. Food and Drug Administration (FDA) issued guidelines for sprout seed decontamination and testing of sprout irrigation waters (FDA, 1999). In three outbreaks of *Salmonella* Enteritidis from 2000 to 2001, it was determined that the contamination was a result of these guidelines not being followed (Mohle-Boetani *et al.*, 2009). Specifically, the seeds were being treated with 11,000 ppm or less sodium hypochlorite rather than the FDA-recommended level of 20,000 ppm (Winthrop *et al.*, 2003; Mohle-Boetani *et al.*, 2009). Additionally, sprout irrigation water at the producer's farm tested positive for *Salmonella*. A survey of California sprout growers confirmed that most alfalfa sprout growers were achieving the 20,000 ppm disinfection guidelines and if followed, disinfection was achieved (Thomas *et al.*, 2003). However, the same survey found that the mung bean sprouts sampled were not achieving the disinfection guidelines and thus mung bean sprouts may represent a larger risk. It was noted in this survey that seeds and calcium hypochlorite were not measured prior to mixing with water which may partially explain why treatment was not fully effective.

In the outbreaks of *Salmonella* Enteritidis due to contaminated mung sprouts, growers and private laboratories, which noted positive results, did not inform public health officials (Mohle-Boetani *et al.*, 2009). Furthermore, the grower continued to use seeds from a contaminated lot. These two points perpetuated the outbreak which could have been interrupted with help from public health officials. However, it is not clearly recommended that growers report positive results to public health officials, which represents a serious gap in food safety.

From 1995 to 2003, no fewer than 10 outbreaks of salmonellosis occurred due to contaminated sprouts. Alfalfa sprouts were the source of two large outbreaks in 1995 and 1996, in which more than 700 people were made ill and one death was reported. Mung sprouts were also a source of *Salmonella* that resulted in 45 reported illnesses in 2000 (Mohle-Boetani *et al.*, 2009). One potential source of contamination to seeds may be contact with birds or rodents during storage or shipping (Mahon *et al.*, 1997). In addition, some seeds may be set or scored in fields upon which animals directly graze and these seeds can become contaminated from animal feces (Brooks *et al.*, 2001). This is the scenario of an outbreak in September of 2008 in which 13 illnesses in Washington and Oregon of *Salmonella* Typhimurium were directly linked to consuming alfalfa sprouts (FDA, 2008b).

Because *Salmonella* can survive for months on seeds (Beuchat and Scouten, 2002) and has the ability to grow during the sprouting process, even a low level of seed contamination can be considered a risk. The growth of *Salmonella* during the sprouting process has been well documented (Jacquette *et al.*, 1996; Gandhi *et al.*, 2001). In fact, under certain sprouting conditions, *Salmonella* can grow as much as 4 log₁₀ units in 48 hours (Fu *et al.*, 2008). However, technological advances in equipment used and in practices during cultivation have been implemented to reduce growth during

TABLE 1. EXAMPLES OF HUMAN OUTBREAKS OF SALMONELLOSIS DUE TO CONTAMINATED PRODUCE

<i>Produce</i>	<i>Salmonella serotype</i>	<i>Year</i>	<i>No. of illnesses</i>	<i>Contamination source^a</i>	<i>Additional reference</i>
Watermelon	<i>Salmonella</i> sp.	1950	6		Blostein 1993
Watermelon	Miami	1954	17		Gayler <i>et al.</i> 1955
Watermelon	Oranienburg	1979	18		CDC, 1979
Watermelon	Javiana	1991	26	Rind contamination	FDA, 2001
Watermelon	Javiana	1993	27	Rind contamination	
Watermelon	Newport	2006	20		
Cantaloupe	Poona	1991	>400		CDC 1991; FDA, 2001
Cantaloupe	Chester	1989–1990	<245	Rind contamination	FDA 2001
Cantaloupe	Saphra	1997	24		Mohle-Boetani <i>et al.</i> 1999
Cantaloupe	Poona	2000	43	Rind contamination	FDA, 2001
Cantaloupe	Poona	2001	50	Rind contamination	CDC, 2002
Cantaloupe	Poona	2001	27	Rind contamination	CDC, 2002
Cantaloupe	Muenchen	2003	58	Rind contamination	
Cantaloupe	Litchfield	2008	51	Rind contamination	
Cantaloupe	Newport	2003	68	Rind contamination	
Honeydew	Litchfield	2007	30		
Honeydew	Enteritidis	1999	82	Ill food handler	
Honeydew, musk, Watermelon					
Honeydew, musk, Watermelon	Poona	2001	23		
Cantaloupe/honeydew					
Mung sprouts	Muenchen	2003	58		
Mung sprouts	Enteritidis	2000	45	Environment at sprouter	Mohle-Boetani <i>et al.</i> 2009
Mung sprouts	Enteritidis	2001	35	Environment at sprouter	Mohle-Boetani <i>et al.</i> 2009
Mung sprouts	Enteritidis	2001	26	Environment at sprouter	Mohle-Boetani <i>et al.</i> 2009
Alfalfa	Anatum	1990	15		
Alfalfa	Stanley	1995	>272	Contaminated seeds	Mahon <i>et al.</i> , 1997
Alfalfa	Newport	1995–1996	>133	Contaminated seeds	Van Beneden <i>et al.</i> , 1999
Alfalfa	Stanely	1995	128		Jaquette <i>et al.</i> , 1996
Alfalfa	Stanely	1996	30		Jaquette <i>et al.</i> , 1996
Alfalfa	Montevideo/Meleagridis	1996	>500	Contaminated seeds	Taormina <i>et al.</i> 1999
Alfalfa	Senftenberg	1997–1998	52	Contaminated seeds	Taormina <i>et al.</i> 1999
Alfalfa	Infantis/Anatum	1997	109	Contaminated seeds	Taormina <i>et al.</i> 1999
Alfalfa	Havana	1998	18	Contaminated seeds	Backer <i>et al.</i> , 2000
Alfalfa	Havana/Cubana/Tennessee	1998	34	Contaminated seeds	Taormina <i>et al.</i> 1999
Alfalfa	Mbandaka	1999	68	Contaminated seeds	Harris <i>et al.</i> , 2003
Alfalfa	Muenchen	1999	99	Contaminated seeds	Proctor <i>et al.</i> , 2001
Alfalfa	Kottbus	2001	31	Contaminated seeds	Winthrop <i>et al.</i> , 2003
Alfalfa	Saintpaul	2003	16		
Alfalfa	Chester	2003	26	Contaminated seeds	
Alfalfa	<i>Salmonella</i> spp.	2004	12		
Alfalfa/clover	Saintpaul	1999	36		
Alfalfa/clover	Typhimurium	1999	112		
Tomatoes	Javiana	1990	174	Water bath	Brooks <i>et al.</i> , 2001
Tomatoes	Montevideo	1993	84	Water bath	Sivapalasingam <i>et al.</i> , 2004

Tomatoes	Baildon	1998–1999	85	Contaminated field by domesticated animals	Cummings <i>et al.</i> , 2001
Tomatoes	Thompson	2000	42	Unknown	Gupta and Crowe, 2001
Tomatoes	Newport	2002	510	Contaminated pond water used for irrigation	Greene <i>et al.</i> , 2008
Tomatoes	Braenderup	2004	561	Food preparation environment?	Gupta <i>et al.</i> , 2007
Tomatoes	Braenderup	2005	84	Contaminated pond water used for irrigation	Gupta <i>et al.</i> , 2007
Tomatoes	Newport	2005	72	(related to 2002 outbreak) Food preparation environment?	Greene <i>et al.</i> , 2005
Tomatoes	Typhimurium	2006	183		FDA, 2006
Tomatoes	Berta	2006	16		
Red Chili peppers	Montevideo	2000	27		
Serrano peppers	Saintpaul	2008	1442	Contaminated irrigation water	FDA, 2008a
Mangoes	Oranienburg	1998	9		Sivapalasingam <i>et al.</i> , 2003
Mangoes	Newport	1999	79	Contaminated processing water	Beatty <i>et al.</i> , 2004
Mangoes	Saintpaul	2001	26		CDC, 1990
Mangoes	Saintpaul	2003	17		
Carrots	Braenderup	1990	111		
Carrots	Typhimurium	2005	8		
Lettuce	Enteritidis	1992	12		
Lettuce	Heidelberg	1993	18		
Lettuce	Braenderup	1994	19		
Lettuce	Thompson	1994	16		
Lettuce	Enteritidis	2003	14		
Lettuce	Newport	2004	97		
Cilantro	Thompson	1999	76		
Basil	Senftenberg	2007	74 total (11 in US)	Contamination at grower?	Campbell <i>et al.</i> , 2001
Mushroom	<i>typhimurium</i> var. Copenhagen	2002	10	Contamination at grower?	Pezzoli <i>et al.</i> , 2008
Mushroom	Heidelberg	2003	65		
Potato	Enteritidis	1991			
Potato	Enteritidis	1998	21		
Potato	<i>Salmonella</i> spp.	2004	16		
Potato	<i>Salmonella</i> spp.	2004	26		
Strawberries	<i>Salmonella</i> Group B	2003	13	Food preparation environment	

^aData source http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm. If no source is given, no source was determined for that outbreak.

sprouting. Among these improvements are using a rotary drum sprouting process and employing frequent irrigation with unrestricted drainage, which has been shown to aid in washing away any pathogen contamination (Fu *et al.*, 2008). In addition, cool water used for irrigation and cool air blowing over the sprouts has also been demonstrated to reduce the rate of pathogen growth.

Even with these improvements, the risk of contamination to products still exists which warrants a need for effective post-harvest treatments. For example, Gandhi and Matthews (2003) demonstrated that combined treatment of seeds with 20,000 ppm calcium hypochlorite followed by 100 ppm chlorine or calcinated calcium during germination and sprout growth did not eliminate *Salmonella* from alfalfa seeds and sprouts. Low doses of irradiation (1 to 2 kGy) were effective at eliminating *Salmonella* and did not adversely affect sensory or nutritional properties of the sprouts (Saroj *et al.*, 2007), but public perception of irradiated food remains suspicious at best (O'Bryan *et al.*, 2008). Each treatment option has some drawbacks, therefore, before any commercial application can be pursued, additional treatments need to be evaluated to determine the optimal treatment to render sprouts safe.

Tomatoes

Salmonella outbreaks due to contaminated produce are usually widely dispersed, which suggests that contamination is occurring early in production, such as in the field or in a processing plant. Contamination of tomatoes early in production may occur by a mechanism described by Zhuang *et al.* (1995). The researchers demonstrated that if dilated plant cells on the surface of a warm tomato are exposed to cold water contaminated with *Salmonella*, the cells of the tomato will rapidly contract and take in the *Salmonella* through openings such as the stem scar. It has also been demonstrated that *Salmonella* inoculated onto the surface of the tomato can attach to the exterior of the tomato and populations can increase with time, depending on the environmental conditions (Iturriaga *et al.*, 2007). Shi *et al.* (2007) reported that many serovars of *Salmonella* can colonize and survive on tomatoes, but that growth was serovar dependent. Serovars Enteritidis, Typhimurium, and Dublin (group D serovars more commonly associated with poultry) were less adapted to grow and persist than Hadar, Montevideo, or Newport (group C serovars). The ability of only some serovars to grow in tomatoes may be a reason why only a few serovars of *Salmonella* are linked to outbreaks. The pH of tomatoes is fairly acidic and ranges from 3.4 to 4.8 depending on the ripeness of the tomato (Wolf *et al.*, 1979). However, even at this pH, Zhuang *et al.* (1995) demonstrated that *Salmonella* Montevideo populations in chopped tomatoes can increase by 1.5 and 2.5 log units when stored for 22 hours at temperatures of 20°C and 30°C, respectively.

Investigations of *Salmonella* outbreaks due to contaminated tomatoes have pinpointed sources including contaminated irrigation water and contaminated wash water (Table 1). One particular outbreak in 2005 was eventually linked to an outbreak that occurred 3 years earlier that was thought to have affected as many as 2500 people (Greene *et al.*, 2008). Through use of pulsed-field gel electrophoresis, isolates from these two outbreaks were shown to be genotypically identical to each other and to *Salmonella* isolated from pond water that was being used for irrigation purposes. No source of *Salmonella* to

the pond water was isolated, but investigators speculated that wild birds, reptiles, or amphibians were a likely source.

Once contaminated, tomatoes may be difficult to clean because the efficacy of chlorine to remove *Salmonella* on tomatoes depends on the location of *Salmonella* on the tomato. *Salmonella* on stem scars and cracks in the skin survive better than *Salmonella* on the smooth skin (Cummings *et al.*, 2001). Furthermore, the ability of *Salmonella* and other foodborne pathogens to internalize into tomatoes and other produce creates a significant challenge of eliminating the bacteria from these foods. Sanitizing treatments are generally ineffective against internalized pathogens because they are physically protected from the chemicals. Like chlorine, treatments such as electrolyzed water or ozonated water were shown to eliminate *Salmonella* from the surfaces of the tomato (Chaidez *et al.*, 2007; Park *et al.*, 2008) but are ineffective at reaching any internalized *Salmonella*. Currently, irradiation is the only effective treatment for eliminating *Salmonella* that is internalized in tomatoes or any other produce because of its ability to penetrate tissues of produce to eliminate internalized pathogens (Saroj *et al.*, 2007). Furthermore, Gram-negative bacteria are very susceptible to even low doses (O'Bryan *et al.*, 2008). However, treatment with irradiation can produce off flavors, colors, and odors and destroy some nutrients (Fan and Sokorai, 2008). As previously discussed, a lack of public approval of irradiation hinders the employment of this method for treating foods (O'Bryan *et al.*, 2008).

Mangoes

Contaminated mangoes have been responsible for several outbreaks of salmonellosis in the United States. Like tomatoes, mangoes can internalize *Salmonella* to the fruit portion via the stem scar (Bordini *et al.*, 2007). However, unlike tomatoes, mangoes have a nearly neutral pH and high sugar content that is conducive to bacterial growth. Bordini *et al.* (2007) showed that any internalized *Salmonella* survives during cold storage (8°C) and replicates at room temperature (22°C).

Imported mangoes typically receive a hot water immersion treatment (46.1°C for 70 to 90 minutes) followed by a cold water treatment (21.1°C for 30 minutes) to control any fly larvae on the fruit (Schirra *et al.*, 2000). If the cold rinse water is contaminated with bacteria, bacteria can contaminate the fruit on the outer surfaces and the inner edible portion. The internalization mechanism of *Salmonella* into the mangoes can be explained by air spaces present in the warm fruit contracting when immersed in the cold water thus drawing the cold water into the fruit (Merker *et al.*, 1999). *Salmonella* internalized into mango was thought to be the source of a salmonellosis outbreak in 1999. In this outbreak, the contamination was traced back to contaminated wash water. The tanks were unenclosed and toads, wild birds, and bird feces were found around the tanks, which were the most likely sources of *Salmonella* (Sivapalasingam *et al.*, 2003).

Like tomatoes, no effective treatment exists to eliminate *Salmonella* from mangoes once the bacteria are internalized. This emphasizes the need for effective treatments of wash water in tanks to remove any *Salmonella* contaminating the outside of the fruit and to kill any *Salmonella* that might be introduced into the wash water. In fact, inadequately chlorinated wash water is thought to be a large contributing factor

to the 1999 and 2001 outbreaks of *Salmonella* in mangoes (Beatty *et al.*, 2004). Research supports the effectiveness of chlorine treatment of the wash water. Soto *et al.* (2007) demonstrated that either chlorine or copper ion treatment of wash water was effective at preventing internalization of *Salmonella* in mangoes. Until research leads to treatments that can consistently eliminate internalized *Salmonella*, effective treatment and control of *Salmonella* in the wash water will be crucial to preventing salmonellosis due to contaminated produce.

Serrano peppers

In April of 2008, an outbreak of *Salmonella* serotype Saint-paul began (FDA, 2008c). There were 57 cases of salmonellosis in Texas and New Mexico with 17 hospitalizations and 30 more cases being investigated in seven other states. Preliminary investigations suggested Roma, plum, or round tomatoes were the source of *Salmonella*. By early June, 145 cases were reported and 23 people were hospitalized across 16 states. Tomatoes remained the suspected cause at this point, but the *Salmonella* causing the outbreak had not been isolated from tomatoes or growers' fields. In mid-July, after a lengthy investigation the FDA determined that tomatoes were not the source of the outbreak. However, by this time 1220 people were sickened in 42 states, the District of Columbia, and Canada. The FDA shifted the warning from consuming raw tomatoes to jalapeno and Serrano peppers. The source of the outbreak was finally identified and reported on July 30, 2008, to be contaminated irrigation water used at a farm in Mexico to water crops of jalapeno and Serrano peppers.

The outbreak due to contaminated Serrano peppers was the largest outbreak of salmonellosis due to fresh produce in the United States. Besides losses incurred due to medical costs and loss of productivity, this particular outbreak included additional costs that tomato growers incurred. Tomato sales plummeted due to the FDA-issued warning against the consumption of tomatoes. Florida growers alone estimated losses of 100 million dollars and were requesting disaster relief funds from the government. The United Fresh Produce Association claims federal agencies caused more than 300 million dollars in losses nationwide. Since tomatoes had been the source of multiple outbreaks in the past and no outbreaks have been recorded from Serrano peppers, tomatoes seemed like the most likely source. However, this outbreak demonstrated that *Salmonella* can potentially contaminate any type of produce.

Minor outbreaks and vegetables as indirect sources

Less extensive outbreaks due to mushrooms, carrots, lettuce, and other produce have been reported. There remains very little information concerning the relatively less prominent outbreaks due to reasons previously discussed in *Produce Outbreaks Due To Salmonella*. In addition, since these vegetables are usually consumed together with other vegetables in salads, it is difficult to distinguish the single vegetable source of the pathogen. Herbs, in particular cilantro and basil, have been implicated in outbreaks. In 1999, cilantro was identified as a probable source of salmonellosis, but the point of contamination was never firmly identified (Campbell *et al.*, 1999). Contaminated basil caused an international outbreak that sickened 19 people in the United Kingdom (Pezzoli *et al.*, 2008). There were also 11 cases in the United States with the

same pulse-type as the *Salmonella* Thompson isolated from the basil but only three of the patients confirmed consuming basil.

In the case of potatoes, potato salads containing mayonnaise are typically thought to be the primary source of infection since mayonnaise contains eggs and eggs are a leading source of *Salmonella* (CDC, 2008a). Therefore, some prepared potato salads that are improperly stored have led to food-borne salmonellosis. If eggs containing *Salmonella* are used and acidic vinegar is not added, the *Salmonella* remains viable in the mayonnaise (Xiong *et al.*, 1999; Morgan *et al.*, 2007). Commercially prepared mayonnaise has a water activity, pH, and salt content that creates a very hostile environment for *Salmonella*. In fact, research has shown that the addition of commercially produced mayonnaise actually inhibits the growth of *Salmonella* in some foods (Doyle, 2005).

Besides the produce linked to outbreaks, *Salmonella* has been identified from a number of produce items purchased at retail. *Salmonella* has been isolated from oranges, chilis, cabbage, artichokes, cauliflower, celery, eggplant, spinach, and zucchini (Harris *et al.*, 2003). This may be due to differences in processing of the produce item post-harvest or the fact that some of the produce items are consumed after cooking. In addition, there may be some intrinsic factors associated with these items that prevents *Salmonella* from growing in the product or modifies virulence properties of *Salmonella*. It has been suggested that the food matrix influences the virulence of pathogens because differences in stresses associated with food matrices can have varying effects on bacterial growth physiology and gene expression (Buchanan and Bagi, 2000; Nutt *et al.*, 2003a). Comparing these produce items with produce that has been implicated in outbreaks may provide valuable information that could be used to develop control strategies for *Salmonella* in produce.

The outbreaks from tomatoes, peppers, and melons that affected hundreds of people across multiple states demonstrate that clean irrigation water is critical to preventing contamination of crops. Contamination of irrigation water can be difficult to trace and sources such as domestic animal manure can pollute irrigation water (Uhlich *et al.*, 2008). If not properly addressed, these sources of contamination can persist and cause continuous outbreaks such as the tomato outbreaks of 2002 and 2005, which were both linked to the same water source but occurred over a 3-year period (Greene *et al.*, 2008). The importance of environmental sources of *Salmonella* and the potential route of contamination to produce are discussed in the following sections.

Environmental Sources of Vegetable *Salmonella* Contamination

Contamination from the environment

Contamination of fresh produce can occur virtually anywhere throughout the production process. Preharvest sources of contamination are likely to be present before the fruits or vegetables are even removed from their natural environments. Many spore-forming bacteria such as *Clostridium botulinum*, *C. perfringens*, and enterotoxigenic *Bacillus cereus* are found in the soil and may be naturally present on some fruits and vegetables (Beuchat and Ryu, 1997). Other types of bacteria or viruses present on fresh produce may be due to direct contact with fecal material or exposure to untreated wastewater used for irrigation. The use of raw animal manure

as fertilizer can increase the presence of microorganisms on fruits and vegetables as well (Crane *et al.*, 1983; Edwards and Daniels, 1992; Solomon *et al.*, 2002; Hutchison *et al.*, 2004). Another source of preharvest contamination that is often overlooked is contact with wild or domestic animals. For example, wild birds are known to potentially harbor bacterial foodborne pathogens including *Campylobacter* and *Salmonella* (Luechtefeld *et al.*, 1980; Keener *et al.*, 2004; Saleha, 2004). Contact with fecal material from these animals can potentially result in the contamination of vegetable crops.

Specific agricultural practices such as the use of animal manure as opposed to chemical fertilizers may be a factor in introducing these foodborne pathogens to fresh produce (Tauxe *et al.*, 1997). Also, the use of contaminated irrigation water is a potential vehicle for transferring *Salmonella* to the surfaces of fresh fruits and vegetables (Steele and Odumeru, 2004). Given the incidence of *Salmonella* as a foodborne pathogen in general and its distribution in a variety of environments, *Salmonella* species causing foodborne disease are of particular concern as produce contaminants.

Foodborne Salmonella in animal sources

A majority of *Salmonella* infections are zoonotic and the type of animal reservoir is a vital factor in understanding the epidemiological pattern of the disease. While poultry is considered the most prevalent and important source of salmonellae (CDC, 2008a), other animals have been shown to harbor this bacterial species as well. Reptiles are known to carry a variety of *Salmonella* serovars including *S. enterica* (Geue and Löschner, 2002). In a report issued by the Centers for Disease Control and Prevention (CDC), it was reported that there were over 50,000 cases of reptile-associated salmonellosis in 1996 (CDC, 1999). Cattle have also been documented to be a source of *Salmonella* as well as *Escherichia coli* (Himathongkham *et al.*, 1999). Cattle infected with *Salmonella* can excrete cells through milk or feces in the absence of any type of clinical symptoms (Losinger *et al.*, 1995). Cattle recovering from a clinical salmonellosis infection may shed the organism typically from 2 to 12 weeks (Galland *et al.*, 2000).

The use of animal manure to fertilize soil is both economically and environmentally beneficial in maintaining soil fertility and quality. However, animal manure used to fertilize grazing grasses of pastures can introduce pathogens that can eventually colonize the gastrointestinal tracts of other food animals and animals from which manure is produced (Holley *et al.*, 2008). Manures used to fertilize crops that contain pathogens can eventually contaminate produce products. There is some competition with soil organisms as well as adverse conditions that can lead to a reduction of pathogens; however, *Salmonella* has been shown to survive in soil samples for 231 days (Islam *et al.*, 2004). Furthermore, Islam *et al.* (2004) suggested that pathogen movement through the soil is likely and they could reach aquifers or surface waters. If these waters are used for irrigation, crop contamination may occur.

An additional factor to consider is the ability of pathogens to become endophytes. Contaminated irrigation water and subsequent internalization of *E. coli* O157:H7 into spinach resulted in a large outbreak causing 204 people to become ill (Anonymous, 2006). The simple recycling of animal wastes can lead to large outbreaks and highly complex epidemiological investigations. Therefore, it is critical to control and

eliminate pathogens from manure regardless of where the manure is applied.

Presence of Salmonella in water

The microbiological quality of irrigation water, regardless of the source, is crucial in maintaining safe food products. Non-wastewater sources that are not normally considered contaminated with waste or fecal material could play a large role in the presence of *Salmonella* and other microorganisms on fresh produce. Major rivers have been used as irrigation sources for many agricultural settings (Assadian *et al.*, 1999; Garcia *et al.*, 2001). The annual bacterial loads of *Salmonella* in these rivers and coastal areas are essential for assessing potential risk (Baudart *et al.*, 2000). *Salmonella* appear to possess the mechanisms to retain viability and successfully survive in these river environments as well. In samples of river water, *Salmonella* cells were shown to be viable even after 31 days of exposure as determined by reculturing and obtaining plate counts (Santo Domingo *et al.*, 2000). Furthermore, *Salmonella* cells exposed to and surviving stresses associated with freshwater laboratory microcosms have been demonstrated to increase the expression of a key virulence gene regulator (Nutt *et al.*, 2003b).

Not only do *Salmonella* cells survive in river-water samples but this may be one of the larger reservoirs harboring viable bacteria. Baudart *et al.* (2000) reported that *Salmonella* Typhimurium was dominant in river water and marine and freshwater sediments. They concluded that the presence of this species in the marine sediments near the river discharge supported the ability of *Salmonella* to survive long-term in a natural environment. In a study by Polo *et al.* (1998), environmental water samples were collected and plated on media selective for *Salmonella* cells. River-water samples yielded the highest proportion of *Salmonella* cells compared to other sampled water sources from beaches and freshwater reservoirs. This raises a major concern given that currently many rivers are used extensively as irrigation sources for fruits and vegetables.

Strategies for Limiting *Salmonella* Contamination of Produce

The produce industry faces unique challenges for eliminating pathogen contamination when compared to other types of foods due to three specific factors related to produce: 1) produce is typically consumed without cooking; 2) produce is usually not packaged; and 3) the ability of pathogens to internalize into produce exists. Due to these factors, addressing prevention of contamination at all stages of production is necessary. However, since bacteria are ubiquitous throughout nature, it is unrealistic to assume that pathogen-free produce can always be achieved.

Contamination intervention strategies such as Hazard Analysis and Critical Control Point (HACCP) that were first implemented for meat products are being designed for produce production. The application of HACCP to minimally processed crops has been reviewed (Hurst, 2006; Leifert *et al.*, 2008). HACCP aims to identify, monitor, and control potential points in processing that might introduce hazards to the finished product. A determination of the critical control points in any production system is a complex procedure that varies with each product and the process being used (Sperber, 1992).

The USDA guide to minimize microbial food safety hazards for fresh fruits and vegetables suggests proactive monitoring of potential critical control points such as microbial testing of agricultural water (USDA, FDA/CFSAN, 1998). However, even with the proper plan and systems in place, it may still be possible for some microbial contamination to occur and it is at that point that corrective actions need to be taken. Corrective actions can involve two activities: 1) determining and fixing the point in the production chain at which contamination was introduced; and 2) determining what to do with the product.

Potentially contaminated products will need to be evaluated to determine if a microbiological risk exists. After which it can be determined if treatments will render the product safe or if the product will have to be discarded. Treatment options to eliminate bacteria from produce are being explored. Even packaging is being designed with specific applications for produce. These challenges producers face and options for addressing problems are discussed in the next sections.

Elimination of pathogens

Treatment of produce poses a particular challenge because consumers demand a fresh and minimally processed product. Furthermore, not all treatments may be equally effective at eliminating different types of bacteria. For these reasons, multiple hurdle or sequential intervention strategies may ultimately be the best option for produce growers and processors. This type of strategy first described by Leistner (1985) employs the use of several treatments in a sequential order to eliminate bacteria. Using this approach, the first treatment can sublethally injure bacteria to the point they are not allowed to recover before the application of a subsequent treatment. Since sublethally injured bacteria are more susceptible to treatments than unstressed cells (Ray, 1986), sequential application of treatments is more effective at killing bacteria than any single treatment. A sequential treatment plan was shown to be effective for decontaminating raw beef (Delmore *et al.*, 1998). A combination of pre-eviseration washing, an acetic acid rinse, and a final wash followed by a second acetic acid resulted in a reduction of coliform counts of nearly 2 logs while washing or acetic acid rinse alone resulted in 0.4 and 1.1 log reductions, respectively. Similarly, Kang *et al.* (2001) reported a combination of hot water rinse, followed by hot air treatment and sequential rinses with lactic acid were more effective at reducing microbial contamination on beef trim than any single treatment.

Using multiple treatments may allow producers to reduce the severity of treatments, which could result in a higher quality product. This approach could be particularly useful for irradiation of foods since high levels of irradiation can produce off flavors and colors, but lower levels may not be fully effective at eliminating bacteria (O'Bryan *et al.*, 2008). For example, a low dose of irradiation alone did not destroy clostridia spores but was sufficient to sensitize spores to further heat treatment (Patterson, 2001). Similarly, the combination of treating carrots with plant oils and packaging in modified atmosphere lowered the minimum doses of irradiation necessary to eliminate *Listeria* from 0.36 to 0.17 kGy (Caillet *et al.*, 2006).

Even if a completely pathogen-free product is achieved after processing, a large problem to the produce industry is the lack of packaging that can help prevent re-contamination

of products following any sanitizing steps. Produce can be exposed to multiple contamination routes following processing during shipping and display in retail markets. Packaging is typically not used for produce but some new technologies are being developed.

Prevention of pathogen recontamination and growth by packaging

Most produce items are sold without packaging, therefore the possibility of contamination is present after any post-harvest treatment. Akins *et al.* (2008) reported that cantaloupe taken from the wash tank exhibited lower microbial counts than cantaloupe taken after conveyor belts in the packaging area of the processing plant. This suggested that the conveyor belts may have contributed to recontamination of the cantaloupe. The risk of contamination at the retail level also exists simply due to the fact that most produce is not packaged and can be subsequently contaminated by handling. Espinoza-Medina *et al.* (2006) reported that 16.7% of workers handling produce were polymerase chain reaction positive for *Salmonella*.

The use of packaging for cut produce not only reduces this risk of contamination but also allows implementing the means to extend shelf-life. The produce industry has employed methods such as modified atmospheric packaging (MAP) to help suppress microbial growth in their products and to extend shelf life. MAP is a commonly used packaging system to suppress growth of microorganisms where produce is packaged in a low level of O₂ that is replaced with N₂ or CO₂ (typically 50% O₂, 30% N₂, and 20% CO₂). This atmosphere can result in a lower pH, which may inhibit the growth of bacteria (Daniels *et al.*, 1985). Evidence has also shown that elevated levels of CO₂ in produce packaging extends the lag phase of bacteria and can help slow its growth (Zagory, 1999). The nature and abundance of the gasses used in MAP affect certain types of organisms in different ways. Lower levels of O₂ allow for the growth of microaerophilic organisms such as lactic acid bacteria while elevated CO₂ levels favor Gram-positive as opposed to Gram-negative bacteria (Brackett, 1987).

However, the capability of these MAP systems may not always yield products that are safe for consumption and are sometimes ineffective in reducing microbial growth. Abdul-Raouf *et al.* (1993) determined that the use of MAP was not effective in decreasing the survival capabilities of *E. coli* O157:H7 on cucumber or shredded lettuce samples. Synergistic treatment approaches have been suggested such as the use of MAP in combination with a disinfectant-containing wash and may be more effective. Jin and Lee (2007) found that MAP alone had no effect on reducing the levels of *Salmonella* on mung sprouts, but the combined treatments of chlorine dioxide wash and MAP reduced *Salmonella* by 3.0 log CFU/g and maintained a reduced level for 7 days.

Edible films have been explored for applications as packaging of produce. The edible film can create a modified atmosphere around the fruit or vegetable that can aid in extending shelf-life (Navarro-Tarazaga *et al.*, 2008). Edible coatings also offer a moisture barrier that can prevent weight loss of the produce (Baldwin, 1997). In addition, coatings can be impregnated with antimicrobials that could improve food safety quality (Vargas *et al.*, 2008). However, problems of

functionality and durability are associated with the use of edible films for produce. Furthermore, any antimicrobials would only be effective against the microorganisms located on the produce surface. Further engineering of edible films for produce application is currently being developed (Vargas *et al.*, 2008).

Conclusions

A considerable challenge remains to ensure safe produce due to a lack of packaging and the possibility of the produce becoming recontaminated after any post-harvest treatment. Ultimately, a multiple hurdle approach may be the most effective strategy for eliminating *Salmonella* from produce. The ability of pathogens to become internalized into fruit or endophytes within plant tissues also poses a considerable challenge for producers. The internalization of pathogens in produce has sparked extensive research efforts towards developing methods to remove pathogens from the tissues. As the molecular basis of the internalization and interactions with plants is currently being investigated becomes better understood this may provide valuable information to develop more effective intervention strategies (Heaton and Jones, 2008).

Wastewater and freshwater contamination by foodborne pathogens can contribute to the eventual contamination of fruits and vegetables irrigated with water sources that come in contact with these sources. The effect of aquatic environments on pathogen survival and incidence may have a subsequent impact on the amount of contamination. The overall focus of future research needs to be directed at environmental points of bacterial contamination in vegetable production including animal and water sources because of the ubiquitous nature of their ecology. Foodborne salmonellae are an ideal group of bacteria to understand how to differentiate the relative importance of these dissemination pathways and optimal methods for control. This should provide considerable insight because *Salmonella* have been extensively studied and are sufficiently well known genetically for molecular methods to be readily applicable.

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