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# The Role of Foods in *Salmonella* Infections

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## 1. Introduction

*Salmonella* is one of the most common causes of foodborne disease worldwide. It also generates negative economic impacts due to surveillance investigation, and illness treatment and prevention. Salmonellosis is a zoonotic infection caused by *Salmonella*; for example, *S. Enterica* causes gastroenteritis, typhoid fever and bacteremia. Transmission is by the fecal-oral route whereby the intestinal contents of an infected animal are ingested with food or water. Human carriers are generally less important than animals in transmission of *Salmonella* strains. A period of temperature abuse which allows the *Salmonella* spp. to grow in food and/or inadequate or absent final heat treatment are common factors contributing to outbreaks. Meat, poultry, egg, dairy products, and fruits and vegetables are primary transmission vehicles; they may be undercooked, allowing the *Salmonella* strains to survive, or they may cross-contaminate other foods consumed without further cooking. Cross-contamination can occur through direct contact or indirectly via contaminated kitchen equipment and utensils. This chapter is a review of the role foods play in *Salmonella* infections and provides an overview of the main food chain-associated *Salmonella* risks.

## 2. *Salmonella* contamination sources in foods

*Salmonella* is found in the environment and the gastrointestinal tract of wild and farmed animals. Animals may become infected with *Salmonella* through environmental contamination, other animals or contaminated feed. Both animals and humans can function as *Salmonella* reservoirs. In addition to sheep, goats, cattle, chickens and pigs, other animals which can become infected with *Salmonella* include geese and other birds, lizards and other reptiles, shellfish, and amphibians such as turtles. Indeed, most *Salmonella* contamination is of animal origin.

Among livestock production systems, *Salmonella* is more frequently isolated from poultry (chicken, turkey, duck, and pheasants) than from other animals (Freitas et al., 2010).

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*Salmonella*-infected animals shed the microorganism in the feces from where it can spread into soil, water, crops and/or other animals. All *Salmonella* serotypes can be harbored in the gastrointestinal tract of livestock. The most common chain of events leading to this foodborne illness involves healthy carrier animals which subsequently transfer the pathogen to humans during production, handling and/or consumption.

*Salmonella* transmission to food processing plants and food production equipment is a serious public health issue. *Salmonella* can enter the food chain at any point: crop, farm, livestock feed, food manufacturing, processing and retailing (Wong et al., 2002). A number of workers handle animals during slaughter and processing, and contamination is possible when *Salmonella* or any other pathogen is present on the equipment or the workers' hands or clothing. Contamination most often occurs during specific slaughter stages: bleeding, skinning (or defeathering in poultry), evisceration (removal of chest and abdomen contents, also known as gutting) and pre-processing carcass handling. Cattle may be asymptotically infected with *Salmonella* and beef can be contaminated during slaughter and processing via gastrointestinal content, and by milk during milking. *Salmonella* Dublin which is highly pathogenic to humans, is strongly associated with cattle (host-adapted). This makes cattle an important target for *Salmonella* control efforts.

*Salmonella* can frequently be isolated from most species of live poultry, such as broilers, turkeys, ducks and geese. Levels in poultry can vary depending on country, production system and the specific control measures in place. Contamination in poultry products can occur at several stages in the slaughter process, be it feces during evisceration or cross-contamination from contaminated products or surfaces on the production line. Particular contamination 'hot spots' in the poultry slaughter process include defeathering, evisceration and cutting; chilling in a water bath reduces the *Salmonella* load but may in turn facilitate cross-contamination (Corry et al., 2002; Fluckey et al., 2003; Northcutt et al., 2003).

Pork and pork products are increasingly recognized as important sources of human salmonellosis (Nielsen and Wegener, 1997). *Salmonella* colonizes pigs on the farm, and pork is then contaminated during slaughter or subsequent processing. Control of *Salmonella* in pork can be implemented on the farm, at slaughter and during processing. Pre-harvest control consists of monitoring *Salmonella* at the herd level, and implementing *Salmonella* reduction measures in infected herds through hygiene, animal separation, feeding strategy and strict control of *Salmonella* in the breeder and growing-finishing pig supply chain.

Until recently, most human *Salmonellosis* cases have been caused by contaminated food animals, but in recent years an array of new food vehicles in foodborne disease transmission has been identified. Foods previously thought to be safe are now considered to be hazardous. These new food vehicles share several features. Contamination typically occurs early in the production process, rather than just before consumption. Consumer preferences and the globalized food market result in ingredients from many countries being combined in a single product, making it difficult to trace the specific contamination source. Many foods also have fewer barriers to microbial growth, such as added salt, sugar or preservatives. Their consequent short shelf life means they are often eaten or discarded by the time an outbreak is recognized. Under these circumstances, efforts to prevent contamination at the source are very important. Fresh produce such as fruits and vegetables have gained attention as transmission vehicles since contamination can occur at any one of the multiple steps in the processing chain (Bouchrif et al., 2009). Factors influencing the rise in salmonellosis outbreaks linked to vegetables include changes in agricultural practices and eating habits, as well as greater worldwide commerce in fresh produce (Collins, 1997).

Contamination with *Salmonella* strains from fresh produce apparently stems mainly from horticultural products. The principal contamination routes are probably use of animal-source organic fertilizers, irrigation with wastewater, humans and other animals (Islam et al., 2004; Natvig et al., 2002). Presence of *Salmonella* in the environment may also lead to contamination in fruits and vegetables because *Salmonella* can survive for long periods in the environment. Multiple pathogenic microorganism sources occur during food packaging, distribution and marketing.

Studies of environmental sources of *Salmonella* contamination indicate that water is an important source, particularly irrigation water containing manure, wildlife feces or sewage effluents (Islam et al., 2004; Reilly et al., 1981). Insects or birds may also transmit *Salmonella* to different foods. Flies are a known *Salmonella* carrier (Greenberg & Klowden, 1972), and can transmit various pathogenic microorganisms, as well as viruses such as polioviruses, coxsackie viruses, infectious hepatitis and anthrax (Ugbogu et al., 2006). Moore *et al.* (2003) mentioned the possibility that *Chironomus* genus insects were direct or indirect vectors of enteric bacteria contamination in water and food.

In general, non-typhoid *Salmonella* is a persistent contamination hazard in all raw foods, including animals, poultry, wild birds, eggs, fruit, vegetables, dairy products, fish and shellfish and cereals.

### 3. *Salmonella* in foods

*Salmonella* spp. are the most common pathogenic bacteria associated with a variety of foods. Although myriad foods can serve as *Salmonella* sources, meat and meat products, poultry and poultry products, and dairy products are significant sources of foodborne pathogen infections in humans. Presence of *Salmonella* spp. in fresh raw products can vary widely (Harris et al., 2003). Frequency usually ranges from 1 to 10 %, depending on a range of factors including organism, farming and/or food production practices, and geographical factors (Harris et al., 2003). Research on *Salmonella* frequency in different countries is extensive, and *Salmonella* serotypes have been isolated in a variety of foods (Table 1). Poultry and egg products have long been recognized as an important *Salmonella* source (Skov et al., 1999); in fact, contaminated poultry, eggs and dairy products are probably the most common cause of human Salmonellosis worldwide (Herikstad et al., 2002). *Salmonella* can contaminate eggs on the shell or internally, and egg shells are much more frequently contaminated than the white/yolk. Furthermore, egg surface contamination is associated with many different serotypes, while infection of the white/yolk is primarily associated with *S. Enteritidis* (Table 1).

Poultry and poultry products are a common foodborne illness vector. Poultry can carry some *Salmonella* serovars without any outwards signs or symptoms of disease. *Salmonella* can be introduced to a flock via multiple environmental sources, such as feed, water, rodents or contact with other poultry. The gastrointestinal tract of one or more birds may harbor *Salmonella*-and, if damaged during slaughter, may contaminate other carcasses. Cross-contamination can also occur from a *Salmonella*-positive flock or contaminated slaughter equipment to the carcasses of a *Salmonella*-free flock, as well as via handling of raw poultry during food preparation. Sufficient heating will eliminate *Salmonella* from contaminated poultry and poultry products.

Pasteurization effectively kills *Salmonella* in milk, but consumption of unpasteurized milk and milk products is a well documented risk factor for salmonellosis in humans.

Inadequately pasteurized milk as well as post-pasteurization contamination of milk and milk products are recognized sources of human disease.

Country	Food	Serotypes	Reference
United States	Papaya	Agona	CDC, 2011
United States	Cantaloupe	Panama	CDC, 2011
United States	Raw milk	Anatum, Cerro, Dublin, Infantis, Kentucky, Mbdanka, Montevideo, Muenster	Van Kessel et al., 2011
United States	Oysters served raw in restaurants	Newport, Mbandaka, Braenderup, Cerro, Muenchen, I:4,12:i:-	Brillhart & Joens, 2011
Mexico	Chili peppers	ND	Castro-Rosas et al., 2011
Mexico	Cheese	Amsterdam, Anatum, Montevideo, Brandenburg, Give, Kiambu, Nyborg, Bredeney, Typhimurium, Meleagridis, Kentucky	Torres-Vitela et al., 2011
China	Beef	Enteritidis, Typhimurium	Yang et al., 2010
Iran	Chicken	Thompson	Dallal et al., 2010
Brazil	Poultry carcass	Enteritidis	Freitas et al., 2010
Turkey	Retail Meat Products	Typhimurium, <i>S. bongori</i> , <i>S. enterica</i> subsp. <i>diarizonae</i>	Arslan & Eyi, 2010
Uruguay	Poultry and Eggs	Enteritidis, Derby, Gallinarum, Panama	Betancor et al., 2010
Mexico	Zucchini squash	ND	Castro-Rosas et al., 2010
Bangladesh	Chick egg	Typhimurium	Hasan et al., 2009
Senegal	Chicken Carcasses and Street-Vended Restaurants	Brancaster, Goelzau, Kentucky, Hadar, Agona, Poona, Bandia, Bessi, Brunei, Hull, Istanbul, Javiana, Magherafelt, Molade, Oxford, Rubislaw, Tamale, and Zanzibar	Dione et al., 2009
United States	Chicken carcasses from retail stores	Kentucky, Hadar, Enteritidis, Braenderup, Montevideo, Thompson, Mbandaka, Agona	Lestari et al., 2009

Country	Food	Serotypes	Reference
United States	Broiler carcasses	Kentucky, Heidelberg, Typhimurium, Typhimurium var. 5-; 4,5,12:I: -; Schwarzengrund, Montevideo, Ohio, Kiambu, Betha, Thompson; 4,12:I: -; Senftenberg, Enteritidis, Worthington, Hadar; 8,(20): - :z6; Mbandaka; 8,(20):I: -; Infantis	Berrang et al., 2009
Republic of Ireland	Retail pork	Typhimurium	Prendergast et al., 2009
Mexico	Parsley, coriander, cauliflower, lettuce, spinach	Typhimurium, Choleraesuis, Gallinarum, Anatum , Agona, Edinburg, Enteritidis, Typhi, Pullorum, Bongor	Quiroz-Santiago et al, 2009
Japan	Imported Seafood	Weltevreden	Asai et al., 2008
Iran	Raw poultry	Enteritidis, Baibouknown	Jalali et al., 2008
Mexico	Hydroponic Tomatoes	Typhimurium, Agona, Thompson, Montevideo, C1 monophasic	Orozco et al., 2008
Australia	Retail Raw Meats	Typhimuriuam, Infantis	Phillips et al., 2008
Turkey	Chicken	Infantis	Cetinkaya et al., 2008
Germany	Sushi from sushi bars and retailers	ND	Atanassova et al., 2008
Vietnam	Pork, beef, chicken, Shellfish	London, Havana, Anatum, Hadar, Albany, Typhimurium	Van et al., 2007
Brazil	Poultry meat	ND	Reiter et al., 2007
New Zealand	Uncooked retail meats	Infantis, Typhimurium, Enteritidis, Brandenburg, 4,5,12:-: -, 4,12:-: -, 4:-:2, 6,7:k:-	Wong et al., 2007
Canada	Chicken nuggets and strips	Heidelberg, Orion, Kentucky, Hadar, Indiana, Infantis, Enteritidis, Mbandaka,	Bucher et al., 2007



Country	Food	Serotypes	Reference
Malaysia	Street food, fried chicken, kerabu jantung pisang, sambal fish, mix vegetables	Biafra, Braenderup, Weltevreden	Tunung et al., 2007
United States	Almonds	35 different serotypes	Danyluk et al., 2007

ND: not determined

Table 1. *Salmonella* serotypes identified in different foods and countries.

*Salmonella* spp. have been isolated from filter feeder seafood species such as oysters, clams and mussels (Table 1). These species acquire their food from the water flowing through their bodies, but also ingest anything else that happens to be in the water. If oceans, lakes and bays are contaminated with fecal matter, the shellfish living in them intake any waterborne pathogens and harbor them in their intestines. The highest potential infection risk is from oysters, since they are most often eaten raw on the half shell. A single raw oyster can contain enough bacteria to cause an infection in the human gut. Mussels and clams pose less of a risk because they are usually steamed, killing *Salmonella* and most other bacteria. The above constitute only a sampling of the principal ways in which animals and animal products cause lead to *Salmonella* infection.

Fresh produce as a possible disease vehicle has become the focus of increasing concern since contamination can occur at multiple steps along the food chain. *Salmonella* is among the most worrisome of the pathogenic microorganisms found in minimally-processed fresh produce (CDC, 2009; Heaton et al., 2008). Bacterial contamination of whole or minimally-processed fresh vegetables can occur at different processing stages (i.e. harvest, trimming, washing, slicing, soaking, dehydrating, blending and/or packaging) (Harris et al., 2003). Produce can also be contaminated with human or animal source pathogens (Beuchat, 2006; Natvig, 2002). *Salmonella* spp. are the most common etiological agent associated with fresh produce related infection in the United States (US). A range of fresh fruit and vegetable products have been implicated in *Salmonella* infection, most frequently lettuce, sprouted seeds, melons and tomatoes (Table 2). *Salmonella* spp. are often isolated during routine surveys of produce such as lettuce, cauliflower, sprouts, mustard cress, endive and spinach (Thunberg et al., 2002); mushrooms (Doran et al., 2005); bean sprouts, alfalfa sprouts, unpasteurized juices and fresh salad fruits and vegetables (CDC, 2009).

In Mexico, *Salmonella* has been isolated from raw vegetables such as alfalfa sprouts (Castro-Rosas and Escartín, 1999), parsley, cilantro, cauliflower, lettuce and spinach (Quiroz-Santiago et al., 2009). It has also been identified from zucchini squash (*Cucurbita pepo*) (Castro-Rosas et al., 2010), and jalapeño and serrano chili peppers (Castro-Rosas et al., 2011). In 2008, 600,000 tons of zucchini were produced in Mexico: 419,768 tons for the domestic market (SAGARPA, 2010) and approximately 200,000 tons for the US market (USDA, 2010). This squash is most commonly consumed cooked in Mexico and other countries, but can be eaten raw (e.g. green salads). In 2009, over 1,981,500 tons of chili peppers were produced in Mexico; of these 613,308 tons were jalapeño peppers and 216,617 tons were serrano peppers (SAGARPA, 2010). These peppers are most commonly consumed raw [e.g. green salads or Mexican sauce (salsa)], both in Mexico and other countries.

We studied the frequencies of coliform bacteria (CB), thermotolerant coliforms (TC), *Escherichia coli* and *Salmonella* in zucchini squash (Castro-Rosas et al., 2010) and jalapeño and serrano peppers (Castro-Rosas et al., 2011). In zucchini squash, infection was detected in 100% of cases for CB, 70% for TC, 62% for *E. coli* and 10% for *Salmonella* spp. Concentration range was 3.8 to 7.4 log CFU/fruit for CB, and <3 to 1000 MPN/fruit for TC and *E. coli*. In serrano chili peppers infection was detected in 100% of cases for CB, 90% for TC, 50% for *E. coli* and 10% for *Salmonella* spp., while in jalapeño peppers frequencies were 100% for CB, 86% for TC, 32% for *E. coli* and 12% for *Salmonella* spp. All *Salmonella*-positive samples were also *E. coli*-positive. For CB, concentration range was 3.8 to 7.9 log CFU/serrano sample and 5.3 to 8.2 log CFU/jalapeño sample, whereas TC and *E. coli* concentrations ranged from <3 to 1100 MPN/serrano and jalapeño samples (Castro-Rosas, et al., 2010; 2011). As is the case with other vegetables consumed raw, zucchini squash, and jalapeño and serrano peppers are potential pathogen vehicles. Sources of pathogenic microorganisms in the field include soil, water, wild and domestic animals, drift and runoff from adjacent farms and manure (Beuchat, 2006; Natvig, 2002). Once harvested and used in food preparation, zucchini squash, jalapeño and serrano peppers are all potential sources of cross contamination with pathogenic microorganisms.

Salmonellosis infection is an increasing problem and recent salmonellosis outbreaks have been associated with a wider variety of vegetables, even those that were not previously considered to imply a risk (e.g. jalapeño peppers; CDC, 2008a). Data on frequency of incidence for pathogenic bacteria such as *Salmonella* are clearly needed for a wide variety of vegetables which are consumed raw. Preventing contamination is vital to avoiding salmonellosis outbreaks, but it is also important to understand the potential survival and growth rates of *Salmonella* on specific substrates such as zucchini, jalapeño and serrano peppers. Our results suggest that both chili peppers and zucchini squash may be significant factors contributing to the endemicity of *Salmonella* in Mexico.

*Salmonella* has been isolated from fruits and vegetables such as cantaloupes, melons, tomatoes, lettuce, and especially alfalfa sprouts (Table 1). These products can become contaminated by several routes, therefore, consumers need to thoroughly wash all fresh foods before consumption to reduce risk of illness from fruits and vegetables. With alfalfa sprouts and lettuce, washing can merely drive bacteria deeper into the lower layers of lettuce leaves or sprouts, so the outside three layers of lettuce leaves need to be removed and sprouts need to be separated before careful washing.

Finally, consumer awareness needs to be promoted that many other foods may carry *Salmonella*, even those not normally thought to be contamination sources. Most users know to handle raw chicken properly and to cook chicken and eggs thoroughly to avoid *Salmonella* contamination. But foods such as almonds, pecans and chocolate can also harbor *Salmonella*. In addition, as the food chain becomes completely global and highly complex, and international trade continues to develop, new foods will surely be linked to salmonellosis outbreaks.

#### 4. *Salmonella* outbreaks

Disease surveillance reports frequently identify poultry, meat and milk products as the main vehicles in salmonellosis outbreaks. However, in recent years foodborne illness outbreaks have been increasingly associated with greater consumption of fresh fruits and vegetables (CDC, 2009). *Salmonella* is responsible for frequent foodborne illness outbreaks in the



developed world, and *Salmonella* outbreaks have been associated with different *Salmonella* serovars (Table 2). Over 2000 *Salmonella* serotypes are known, but only a small fraction of these are commonly associated with foodborne illness. Which serotypes cause illness is influenced by serotype geographical distribution and serovar or strain pathogenicity. In the US, *Salmonella* Typhimurium has been considered the principal causative agent of foodborne salmonellosis, but both *S. Typhimurium* and *Enteritidis* have been increasingly identified in foodborne salmonellosis since the 1980s (Table 2); the exact cause of the predominance of these *Salmonella* serotypes is not yet clearly understood.

Most developed countries have laboratory-based *Salmonella* infection surveillance programs, and many countries have systems for recording outbreaks and notification systems where clinicians submit data on patients with *Salmonella* infections to national public health institutions. Official *Salmonella* infection numbers are usually derived from laboratory-based surveillance in which clinical microbiology laboratories report positive findings and, in some countries, submit *Salmonella* isolates to national reference laboratories for serotyping and other characterization. These data are necessary for measuring trends over time and detecting outbreaks. However, official figures do not quantify the burden of illness, and degree of surveillance differs between countries. Moreover, reported incidence is a composite measure of several factors, including true *Salmonella* infection incidence, the health-care seeking behavior of patients with gastroenteritis, and the likelihood that the physician requests a stool culture. Furthermore, access to laboratories and microbiological methods varies widely, as does the precision of findings reported to public health authorities. Finally, comparisons between different geographical areas can be difficult because public health jurisdictions with a tradition of active case-searching as part of outbreak investigations or extensive testing of contacts of known patients or food-handlers are likely to report higher numbers of infections than jurisdictions with only passive surveillance. As a result, the precise incidence of *Salmonella* food poisoning in all countries is not known, since small outbreaks often remain unreported.

*Salmonella* spp. and *S. Typhi* infections are endemic in many developing countries. In Mexico, there were 709,278 salmonellosis cases and 228,206 typhoid fever cases reported from 2004-2009 (Secretaría de Salud, 2011). In addition, *S. Gaminara* and *S. Montevideo* have been associated with several cases of human illness in Mexico (Gutiérrez-Cogco et al., 2000). A certain proportion of salmonellosis and typhoid fever cases in Mexico may be associated with consumption of raw vegetables exposed to fecal contamination, probably due to the continued but limited practice of irrigating vegetable crops with untreated wastewater.

Centers for Disease Control and Prevention (CDC) data for the US indicate that over 40,000 salmonellosis cases occur annually, with about 500 resulting deaths. As is the case for staphylococcal gastroenteritis, the largest salmonellosis outbreaks typically occur at banquets or similar functions. However, the two largest recorded salmonellosis outbreaks occurred under rather unusual circumstances. The largest occurred in 1994 and involved over 224,000 cases in 41 states. The serovar was *S. Enteritidis* and the vehicle food was ice cream produced from milk transported in tanker trucks which had previously hauled liquid eggs. The second largest occurred in 1985 and involved nearly 200,000 cases. *S. Typhimurium* was the etiological agent and the vehicle was 2% milk produced by a single dairy plant in Illinois. The third largest outbreak occurred in 1974 on the Navajo Indian Reservation, when 3,400 persons became ill with the *S. Newport* serovar. Human carriers are generally less important than animals in transmission of salmonellosis. Human

transmission can occur if hands contaminated with infected fecal matter come in contact with food which is then consumed without adequate cooking, often after an intervening period in which microbial growth occurs. Exactly this chain of events led to a major outbreak affecting an international airline in 1984. A total of 631 passengers were infected after eating food containing an aspic glaze prepared by a food service worker who returned to work after a bout of salmonellosis but was still excreting *Salmonella* Enteritidis PT4. The serotype Typhimurium has participated in most recent outbreaks, although it is likely that this serotype's involvement in salmonellosis cases worldwide is far greater than reported. *Salmonella* surveillance sensitivity may vary widely between countries but it is still crucial to identifying trends and detecting outbreaks. Surveillance which includes serotyping is particularly useful for this purpose. Available data suggest that the incidence of *Salmonella* infections has increased over the last twenty years, that new *Salmonella* serotypes often emerge in several countries at near the same time, and that multi-state or international outbreaks call for a coordinated response. In response, several national and international networks currently address the problem of emerging *Salmonella* infections. An important objective in preventing *Salmonella* outbreaks is improvement and enhancement of surveillance, including serotyping.

Country	Food vehicle	Serotypes	Number of cases	Reference
United States	Papaya	Agona	99	CDC, 2011a
United States	Alfalfa sprouts and spicy sprouts	Enteritidis	25	CDC, 2011b
United States	Turkey Burgers	Hadar	12	CDC, 2011c
United States	Cantaloupe	Panama	20	CDC, 2011d
United States	Alfalfa sprouts	I 4,[5],12:i:-	140	CDC, 2011e
Denmark	Salami	Typhimurium	20	Kuhn et al, 2011
England	Sandwiches and prepared salads	Typhimurium	179	Boxall et al., 2011
Australia	Raw egg mayonnaise	Typhimurium	87	Jardine et al, 2011
Ireland, United Kingdom (England, Wales, Northern Ireland, Scotland), France, Luxembourg, Sweden, Finland, Austria	Pre-cooked meat products	Agona	163	Nicolay et al., 2011
South Africa	Food served in a school	Enteritidis	18	Niehaus et al., 2011

Country	Food vehicle	Serotypes	Number of cases	Reference
Japan	Boxed lunches	Braenderup	176	Mizoguchi et al., 2011
England	Multiples foods	Enteritidis	63	Janmohamed et al., 2011
United States	Shell Eggs	Enteritidis	1,939	CDC, 2010a
United States	Cheesy chicken rice frozen entrée	Chester	44	CDC, 2010b
United States	Frozen mamey fruit pulp	Typhi	9	CDC, 2010c
United States	Alfalfa Sprout	Newport	44	CDC, 2010d
United States	Red and Black Pepper/Italian-Style Meats	Montevideo	272	CDC, 2010e
United States	Potato salad	Schwarzengrund, Typhimurium	9	CDC, 2010f
United States	Cilantro and chicken meat	Montevideo	58	Patel et al, 2010
Netherlands	Fresh fruit juice	Panama	33	Noël et al., 2010
France	Dried pork sausage	4,12:i:-	90	Bone et al., 2010
China	Water	S. Paratyphi A	267	Yang et al., 2010
United Kingdom	Raw bean sprouts	Bareilly	231	Cleary et al., 2010
Netherlands	Raw or undercooked beef products	Typhimurium	23	Whelan et al., 2010
Australia	Dessert containing raw egg	Typhimurium	20	Reynolds et al., 2010
New Zealand	Watermelon	Typhimurium	15	McCallum et al., 2010
Spain	Infant formula	Kedougou	42	Rodriguez-Urrego et al., 2010
United States	Alfalfa Sprouts	Saintpaul	228	CDC, 2009a
United States	Peanut butter	Typhimurium	529	CDC, 2009b
United States	Unpasteurized orange juice	Typhimurium and Saintpaul	152	Jain et al., 2009
United States	Vegetable-coated ready-to-eat snack food	Wandsworth, Typhimurium	69	Sotir et al., 2009
Australia	Eggs	Typhimurium	22	Dyda et al., 2009
Australia	Bread dumpling			
Australia	loaf prepared with eggs	Enteritidis	8	Much et al., 2009
Australia	Papaya	Litchfield	26	Gibbs et al., 2009

Country	Food vehicle	Serotypes	Number of cases	Reference
Denmark, Norway and Sweden	Pork meat and pork products	Typhimurium	41	Bruun et al., 2009
Australia	Eggs	Typhimurium	19	Slinko et al., 2009
Mauritius	Marlin mousse	Typhimurium	53	Issack et al., 2009
Pakistan	Drinking water	<i>S. typhi</i>	300	Farooqui et al., 2009
France	Cheese made from raw milk	Montevideo	23	Dominguez et al., 2009
France	Goat's cheese	Muenster	25	Van Cauteren et al., 2009
Denmark	Pasta salad with pesto	Anatum	At least 4	Pakalniskiene et al., 2009
Netherlands	Hard cheese made from raw milk	Typhimurium	224	Van Duynhoven et al., 2009
Australia	Chocolate mousse	Typhimurium	8	Roberts-Witteveen et al., 2009
United States	Jalapeño peppers	Saintpaul	at least 1,442	CDC, 2008a
United States	Frozen Pot Pies	I 4,5,12:i:-*	401	CDC, 2008b
United States	Fruit salad	Litchfield	30	CDC, 2008c
United States	Unpasteurized Mexican-style aged cheese	Newport	85	CDC, 2008d
England and Wales	Fresh basil	Senftenberg	32	Pezzoli et al., 2008
Norway	Rucola lettuce	Thompson	21	Nygård et al., 2008
Bulgaria	Minced meat	Typhimurium	22	Pekova et al., 2008
Switzerland	Soft cheese	Stanley	82	Pastore et al., 2008
Denmark	Pork products	Typhimurium	1,054	Ethelberg et al., 2008
Japan	Snapping turtle	Typhimurium	4	Fukushima et al., 2008
Ireland	Meat products	Agona	119	O'Flanagan et al., 2008

Table 2. Recent reported *Salmonella* outbreaks, including country (ies) affected, food vehicle and serovar.

## 5. Interaction of *Salmonella* with foods

*Salmonella* serotypes can grow and survive on a large number of foods (Harris et al., 2003). Their behavior in foods is controlled by a variety of environmental and ecological factors, including water activity, pH, Eh, chemical composition, the presence of natural or added antimicrobial compounds and storage temperature; as well as processing factors such as heat application and physical handling. For example, optimum pH for growth in *Salmonella* is approximately neutral, with values  $> 9.0$  and  $< 4.0$  being bactericidal. Minimum growth in some serotypes can occur at pH 4.05 (with HCl and citric acids), although this minimum can occur at pH as high as 5.5, depending on the acid used to lower pH (Harris, et al., 2003). Growth in *Salmonella* can continue at temperatures as low as 5.3 °C (*S. Heidelberg*) and 6.2 °C (*S. Typhimurium*), and temperatures near 45 °C (temperatures  $\geq 45$  °C are bactericidal). In addition, available moisture (aw) inhibits growth at values below 0.94 in neutral pH media, although higher aw values are required as pH declines to near the minimum growth values (Harris, et al., 2003).

Extensive data is available on the effects of individual environmental factors on *Salmonella* strains, but the effects of their interactions are not as well understood. Parish et al. (1997) determined survival for several *Salmonella* serotypes in orange juice. To achieve a 6 log reduction in *Salmonella* serotypes, orange juice (pH 3.5) had to be stored at 4 °C for 15-24 days. A similar reduction took 43-57 days when the orange juice was at pH 4.1 and 4 °C. Using apple juice, Uljas & Ingham (1999) demonstrated that *S. Typhimurium* DT104 could be reduced by at least 5 log units at pH 3.3 after storage at 25 °C for 12 hours or at 35 °C for 2 hours. These treatments did not achieve a 5 log reduction in *E. coli* O157. At pH 4.1, a 5 log reduction in *S. Typhimurium* DT104 was produced by storage at 35 °C for 6 hours in the presence of 0.1% sorbic acid or by a combination of storage at elevated temperature (25 °C for 6 hours or 35 °C for 4 hours) followed by a freeze/thaw cycle without sorbic acid (Uljas & Ingham, 1999). In the field, the physical environment of vegetable surfaces is considered to be inhospitable for growth and survival of *Salmonella* (for example, temperature and humidity fluctuations, and ultraviolet light) (Dickinson, 1986). Environmental conditions, however, can greatly influence bacterial populations; the presence of free moisture on vegetable surfaces from precipitation, dew or irrigation can promote survival and growth of bacterial populations (Shaper et al., 2006). Certain conditions such as sunlight, particularly shorter ultraviolet wavelengths, can damage bacterial cells (Shaper et al., 2006); selection therefore occurs for bacteria with adaptations to stressful conditions. Microorganisms' ability to survive on plants depends on the environmental, physicochemical and genetic features of the plant and specific properties (Shaper et al., 2006). Many microorganisms have developed mechanisms to attach to, survive and/or grow in microniches on different vegetables (Shaper et al., 2006). For instance, surface moisture on vegetables may provide a protective environment for *Salmonella* strains. On vegetable surfaces, microorganisms interact in aggregates and may compete for the limited nutrients available in microniches at the junction of epidermal cells, where water accumulates, cuticular waxes are less dense and nutrients are more available than in other sites (Shaper et al., 2006). Free water in the surface apertures of vegetables (e.g. stomata) constitutes a water channel connecting a plant's apoplast with its external environment. Microorganisms can enter vegetables through these water channels in various ways. Once internalized, the microorganisms are protected from environmental stress (Shaper et al., 2006). Survival of pathogenic microorganisms on or in raw produce is



also dictated by its metabolic capabilities. However, the manifestations of these capabilities can be greatly influenced by intrinsic (e.g. vegetable moisture surface) and extrinsic ecological factors naturally present in the raw produce or imposed at one or more points during production, processing and distribution (Harris et al., 2003). *Salmonella* strains may be able to enter a viable but nonculturable state (VBNC) on the surface of fruit and vegetables, resulting in underestimation of viable population size by direct plating on culture medium. Brandl and Mandrell (2002), suggested that *S. Thompson* may enter into a VBNC state on *Cilantro phyllosphere* due to exposure to dry pre-harvest conditions on the plant surface. Improved understanding of microbial ecosystems on the surface of foods such as raw fruits and vegetables would be extremely useful in developing strategies to minimize contamination, prevent pathogen growth, and kill or remove pathogens at different stages in production, processing, marketing and preparation for consumption. Food ecosystems are extremely diverse and complex. *Salmonella* survival and/or growth on foods are influenced by the organism, produce item and environmental conditions in the field and post-harvest, including storage conditions. For many years, the interaction of *Salmonella* with animal hosts and animal-origin foods has received intense attention. In contrast, little research has been done on the interaction between *Salmonella* spp. and fruits and vegetables, and more specifically on its frequency and behavior in fruits and vegetables which may pose a special risk to humans [e.g. radish root (*Raphanus sativus*), beetroot (*Beta vulgaris* var. *conditiva*), jicama (*Pachyrhizus erosus*), loroco (*Fernaldia pandurata*), prickly pear (*Opuntia* spp.), zucchini squash (*Cucurbita pepo*), chili peppers (Jalapeño and Serrano peppers) and others]. It is particularly urgent to study fruits and vegetables not previously considered health hazards and those with the potential to function as pathogen microorganism vehicles but are as yet unidentified.

In a recent *Salmonella* outbreak in the US, jalapeño and serrano peppers were the food vehicle and the isolated serovar was Saintpaul (CDC, 2008). It affected at least 1,442 persons in 43 states, the District of Columbia and Canada, and was traced back to distributors in the United States which had received produce grown and packed in Mexico. The outbreak strain was isolated from samples of jalapeño peppers collected from a US warehouse and a patient's home, as well as from samples of serrano peppers and water collected from a farm in Mexico. We have studied the behavior of *Salmonella* serotypes in zucchini squash and chili peppers. In zucchini, we tested the behavior of four *Salmonella* serotypes (Typhimurium, Typhi, Gaminara and Montevideo) and a cocktail of three *Escherichia coli* strains on whole and sliced zucchini squash at  $25 \pm 2$  and  $3-5$  °C. No growth was observed for any of the tested microorganisms or the cocktail on whole fruit stored at  $25 \pm 2$  or  $3-5$  °C. After 15 days at  $25 \pm 2$  °C, the tested *Salmonella* serotypes had decreased from an initial inoculum level of 7 log CFU to  $<1$  log and at  $3-5$  °C they decreased to approximately 2 log (Figure 1). Among the *E. coli* strains, survival was significantly higher than for the *Salmonella* strains at the same times and temperatures: after 15 days at  $25 \pm 2$  °C, *E. coli* cocktail strains had decreased to 3.4 log CFU/fruit and at  $3-5$  °C they decreased to 3.6 log CFU/fruit (Figure 1). The observed differences in survival between the *Salmonella* and *E. coli* strains on zucchini squash fruit could be due to factors such as the area inoculated, fruit ripeness and physical and chemical characteristics of the studied fruit and strains. Different strains of *E. coli* O157:H7, *Pseudomonas*, *Salmonella*, and *Listeria monocytogenes* attach to different regions of cut lettuce leaves, indicating different and specific attachment mechanisms among different species or strains (Takeuchi et al., 2000).

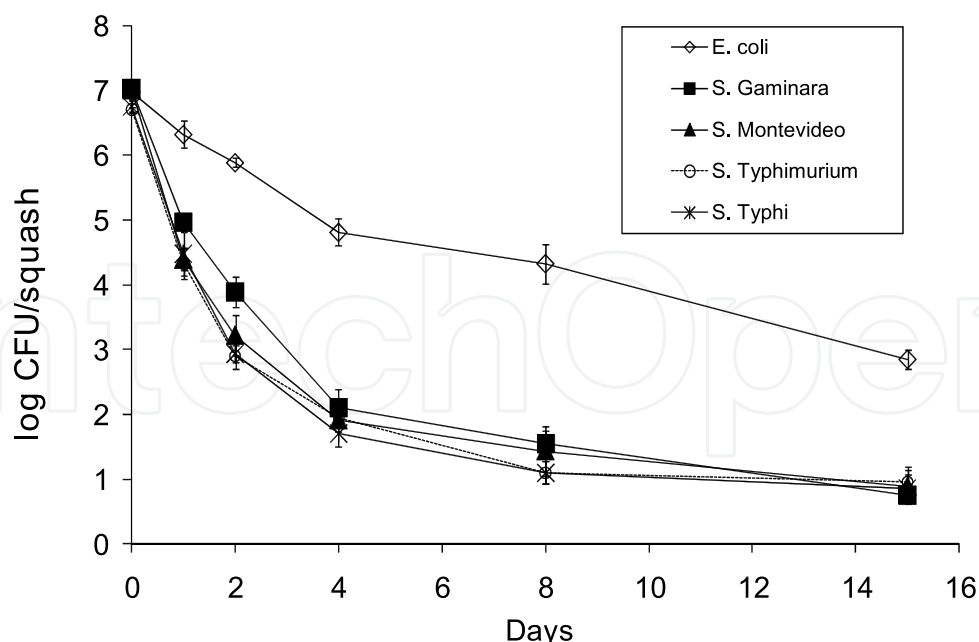


Fig. 1. Behavior of 4 *Salmonella* serotypes and *E. coli* on zucchini squash at  $25\pm 2$  °C (Castro-Rosas et al., 2010).

When inoculated onto zucchini squash slices and incubated at  $25\pm 2$  °C, the studied *Salmonella* and *E. coli* strains grew (Figure 2). After a short lag period (approx. 4 h), the *Salmonella* and *E. coli* populations increased from 2 log to 6 log CFU/slice at 24 h, and the *E. coli* strains increased a further 1 log CFU by 72 h. Initial *Salmonella* and *E. coli* inocula levels were close to that of Aerobic Plate Count bacteria (APC) in the studied zucchini squash fruit (approx. 2.5 log CFU/slice), and the APC growth rate (7.6 log CFU/slice by 24 h; 8.9 log CFU/slice by 72 h) was comparable to the studied strains (Figure 2). The behavior of *Salmonella* under these conditions does not differ greatly from that of *Salmonella* strains in other foods. For instance, *S. Typhimurium* inoculated in shredded cooked beef and stored at 20 °C/8 h, increased from 2.3 to 3.4 log CFU/g (16), while after 22 h incubation on sliced tomatoes *S. Montevideo* increased by ca. 1.5 log CFU/g at 20 °C and 2.5 log CFU/g at 30 °C (Zhuang et al. 1995).

Under refrigeration (3-5 °C), growth in the *Salmonella* serotypes and *E. coli* strains was inhibited (Figure 4): bacterial concentration at 5 days was essentially similar to initial inocula levels. Nonetheless, survival of even a small concentration of *E. coli* and/or *Salmonella* under refrigeration poses a serious health hazard to consumers since salmonellosis outbreaks have been reported as originating in different foods at low pathogen concentrations (Greenwood and Hopper, 1983).

In a separate study, we tested the growth behavior of the same four *Salmonella* serotypes and three *E. coli* strains at the same temperatures ( $25\pm 2$  and 3-5 °C) on whole and sliced jalapeño and serrano peppers, as well as in a blended chili pepper sauce (Castro-Rosas et al., 2011). The sauce was an aqueous suspension containing mixed peppers, tomatoes, coriander, onion and salt (NaCl) in specific proportions. Both types of microorganisms exhibited similar behavior on/in the serrano and jalapeño peppers. No growth was observed in rifampicin-resistant *Salmonella* and *E. coli* strains on the surface of whole serrano and jalapeño peppers stored at  $25\pm 2$  or 3-5 °C. After 6 days at  $25\pm 2$  °C, the tested *Salmonella* serotypes and *E. coli* had decreased from an initial inoculum level of 5 log CFU to 1 log on the serrano peppers and to 2.5 log on the jalapeño peppers (Figure 3). At 3-5 °C they decreased to approximately 1.8 log in

the serrano peppers and to 1.2 log on the jalapeño peppers. In contrast, when inoculated onto slices of both peppers and into the blended sauce, the *Salmonella* serotypes and *E. coli* grew: after 24 h at 25±2 °C, both bacteria types had grown to approximately 4 log CFU on the slices and 5 log CFU in the sauce (Figures 4-5). Bacterial growth was inhibited at 3-5 °C. In summary, the four tested *Salmonella* serotypes can survive on whole or sliced zucchini squash, serrano and jalapeño peppers and in sauce made of raw chili peppers, indicating them to be effective transmission vehicles and potential public health threats.

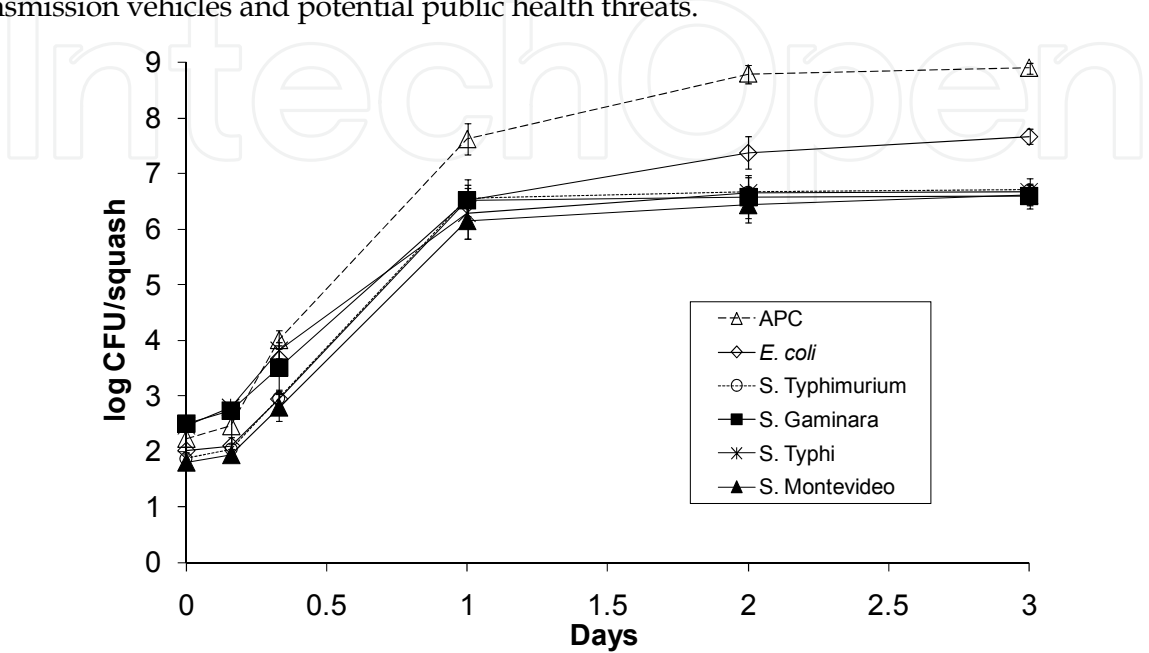


Fig. 2. Behavior of 4 *Salmonella* serotypes, *E. coli* and Aerobic Plate Count on zucchini slices at 25±2 °C (Castro-Rosas et al., 2010).

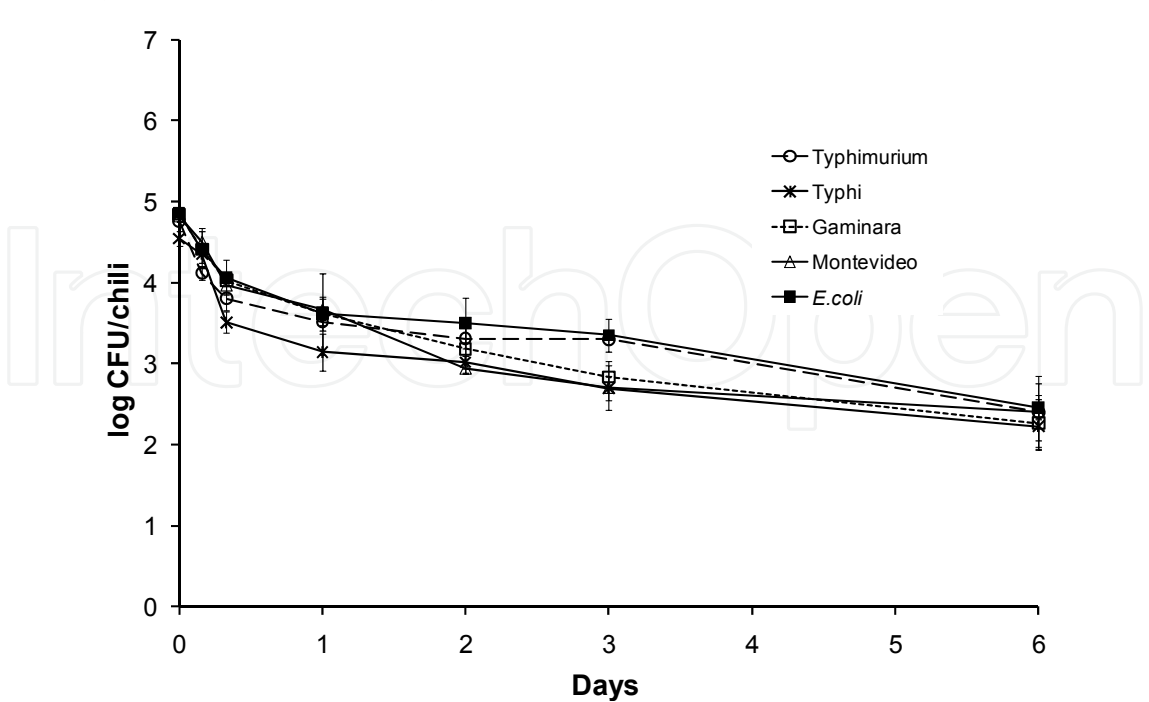


Fig. 3. Behavior of 4 *Salmonella* serotypes and a cocktail of three *E. coli* strains on whole jalapeño peppers at 25±2 °C (Castro-Rosas, 2011).

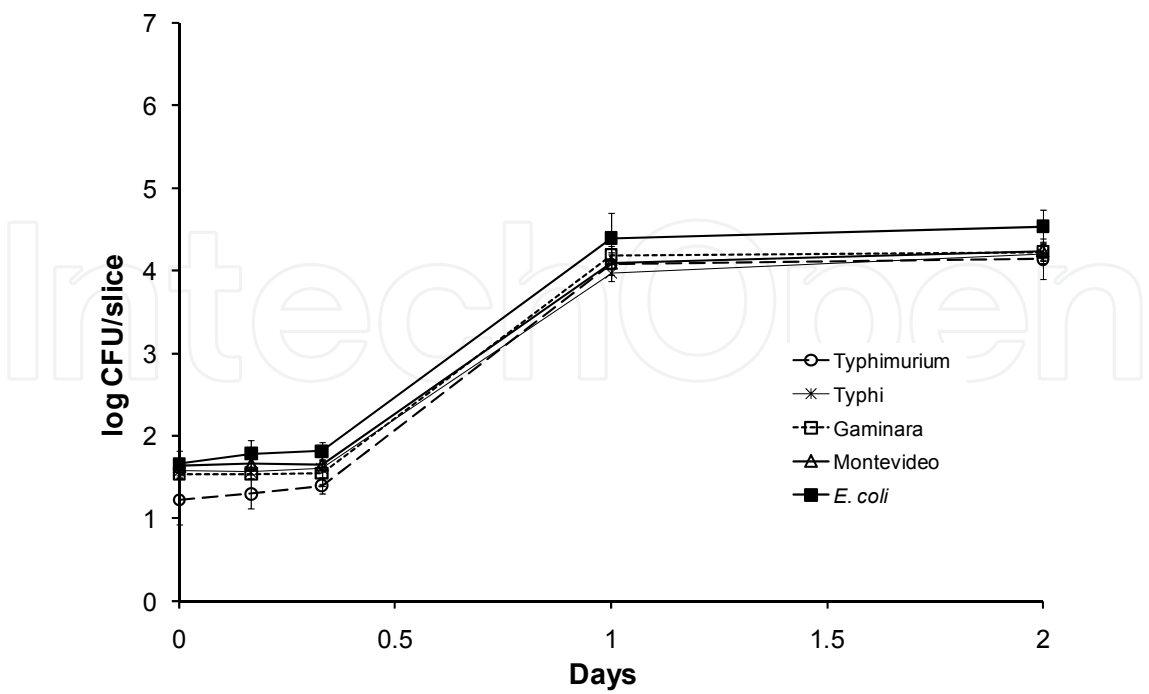


Fig. 4. Behavior of 4 *Salmonella* serotypes and a cocktail of three *E. coli* strains in jalapeño peppers slices at 25±2° C (Castro-Rosas, 2011).

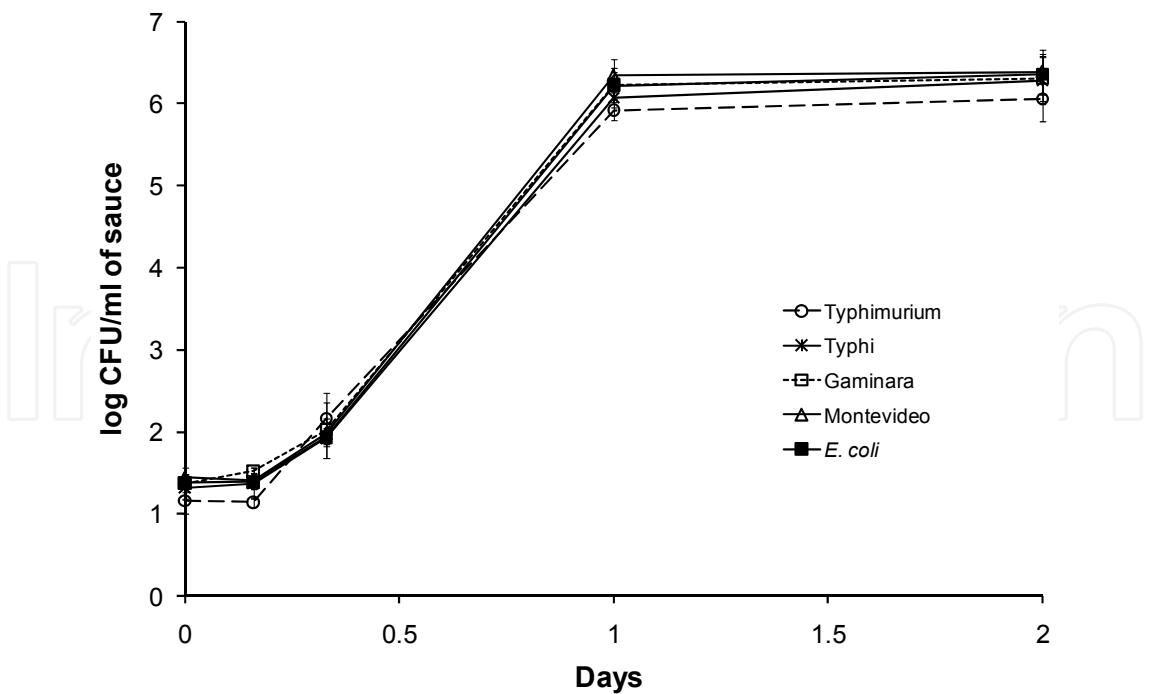


Fig. 5. Behavior of 4 *Salmonella* serotypes and a cocktail of three *E. coli* strains in a chili pepper sauce at 25±2° C (Castro-Rosas, 2011).

## 6. Conclusion

Food is clearly a major *Salmonella* infection vehicle. This vital role in salmonellosis outbreaks calls for strict measures to minimize transmission, such as appropriate animal husbandry and agriculture practices, protection of feeds and water from contamination, adequate waste disposal methods and an overall effort to maintain a clean environment around food from farm to fork. Additionally, much of the risk posed by *Salmonella* can be mitigated through proper handling and correct food safety practices, including thorough washing and disinfection, prevention of pre-consumption, human-borne contamination during preparation and storage, leftovers disposal, cooking before consumption and refrigerated storage (3-5 °C). Continuous monitoring and generation of data on *Salmonella* and salmonellosis outbreaks, and improved surveillance measures are also vital to controlling this public health hazard. A deeper understanding of *Salmonella* and its behavior in foods is still needed to ensure food safety and quality.

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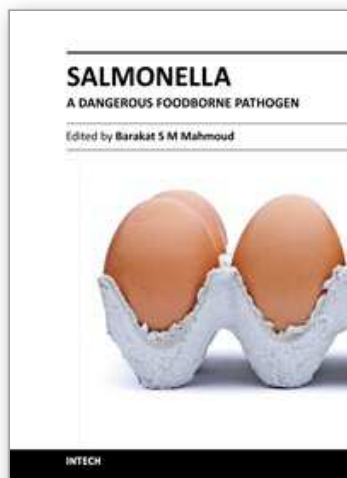
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## **Salmonella - A Dangerous Foodborne Pathogen**

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More than 2,500 serotypes of Salmonella exist. However, only some of these serotypes have been frequently associated with food-borne illnesses. Salmonella is the second most dominant bacterial cause of food-borne gastroenteritis worldwide. Often, most people who suffer from Salmonella infections have temporary gastroenteritis, which usually does not require treatment. However, when infection becomes invasive, antimicrobial treatment is mandatory. Symptoms generally occur 8 to 72 hours after ingestion of the pathogen and can last 3 to 5 days. Children, the elderly, and immunocompromised individuals are the most susceptible to salmonellosis infections. The annual economic cost due to food-borne Salmonella infections in the United States alone is estimated at \$2.4 billion, with an estimated 1.4 million cases of salmonellosis and more than 500 deaths annually. This book contains nineteen chapters which cover a range of different topics, such as the role of foods in Salmonella infections, food-borne outbreaks caused by Salmonella, biofilm formation, antimicrobial drug resistance of Salmonella isolates, methods for controlling Salmonella in food, and Salmonella isolation and identification methods.

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