FAO/MIRCEN

Mangalore, India

Iddya Karunasagar
Son Radu
William Burkhardt
Cristobal Chaidez Quiroz
Indrani Karunasagar
K.V. Lalitha
Myat Myat Htwe
Mohammed A. Hamod and
G.B.Nair

HAZARD CHARACTERIZATION

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Hazard Characterization

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Pathogen, host, and food matrix factors

Characteristic of the pathogen

Salmonella, a Gram negative bacterium, are a common cause of food borne illnesses worldwide but seafood as a source of outbreaks is relatively low. Salmonella infections show complex pathogenesis. A variety of fimbrial adhesions are involved in initiation of contact to host cells (Hensel, 2004). Many of the virulence phenotypes of S. enterica are encoded by genes on the pathogenicity islands (PAI), which are referred to *Salmonella* pathogenicity islands or SPI. At present 12 different SPI have been described. While the roles in pathogenesis of some SPI are well defined, the function in virulence of many genes within SPI is not understood (Hensel, 2004). The O side chains of the lipopolysacchride molecules have also been shown to affect invasiveness and enterotoxin production (Murray, 1986). Other factors that affect the ability of the organism to cause disease include the presence of cytotoxins and diarrhoeagenic enterotoxins. The enterotoxin is released into the lumen of the intestine and results in the loss of intestinal fluids (D'Aoust, 1991). Antimicrobial resistant strains are somewhat more virulent than susceptible strains, in that, they cause more prolonged or more severe illness than do antimicrobial susceptible strains (Travers and Barza, 2002).

Serotyping is an important surveillance tool and more than 2500 serotypes are currently recognized. Ninety nine percent of the human infections are due to *Salmonella enterica* which has about 1500 serotypes. Two broad categories of *Salmonella enterica* infections are recognized and these are typhoidal and non typhoidal (NTS). Based on an analysis of globally reported food borne outbreaks, the NTS serotypes are most often encountered in human infections followed by

Typhimurium (Greig and Ravel, 2009). Major serovars associated with seafood borne illness were Enteritidis, Typhimurium and other *S. enterica*. There are regional differences in serovars encountered (Table 1).

A different picture emerges when we look at serovars prevalent in seafood (Table 1). Analysis of 11,312 imported seafood and domestic seafood over a nine year period (1992-1998), S. Weltevreden was the most common serotype followed by Senftenberg and Lexington. Among the serotypes involved in human infection, Enteritidis ranked fifth and Typhimurium ranked twelfth (Heinitz et al., 2000). In India, data from the National Reference Centre for Salmonella indicate that sea foods account for 3.9% of the *Salmonella* isolated and follow poultry (31%) and animals (13.9%). In seafood in India, the commonest serotype encountered was S. Worthington followed by Weltevreden. This pattern of dominance of S. Weltevreden seems to be reflected in reports from other countries in Southeast Asia.

Salmonella serotypes are closely related genetically yet differ significantly in their pathogenic potentials. It is reported that *Salmonella* strains isolated from most of the clinical cases appear to be different from those found in shrimp and other aquaculture products resulting in the conclusion that these seafood constitutes a very low risk to public health (Feldhusen, 2000). However, this might not be true for all countries for example S. Weltevreden which is a common isolate from shrimp culture environments and shrimp product is also the most common serotype involved in human infection in Thailand (Bangtrakulnonth et al., 2004), Vietnam (Phan et al., 2005) and Malaysia (Yassin et al., 1995). Maybe this could be attributed to the consumption of raw seafood particularly shrimps in salads kept at room temperature leading to multiplication of the pathogen.

Characteristic of the host

Salmonella species are a leading cause of food borne illnesses worldwide and their incidence is dependent upon a variety of factors including host susceptibility. In general, the host factors that can affect outcome of exposure to *Salmonella* or any food borne pathogen by ingestion include age, nutritional status, socio economic and environmental factors, immune status and underlying diseases. This susceptibility can often be associated with socio-economic status and demography.

Age and the general health of the exposed population are factors that should be considered when assessing the susceptibility of the host to infection. In addition to age, the immunological condition of the host apparently plays a significant role in disease. Children who have immature immune systems and people who are immuno-compromised account for the majority of all reported laboratory confirmed cases of Salmonellosis. It has been noted children who have more neutral stomach pH are more

susceptible due to the acid susceptibility of these pathogens. While host stomach pH can affect host susceptibility, the matrix in which the pathogen is consumed may promote/protect the agent from low pH in the stomach. Following resolution of the acute phase, excretion of *Salmonella* ceases within several weeks, although a carrier state may evolve.

Characteristic of the sea food matrix

Gastric acidity (pH 2.0) is considered an important defense against food borne pathogens. Though normally *Salmonella* grow at neutral pH, they have complex survival strategies that might facilitate their ability to tolerate pH fluctuations during pathogenesis. Most aquaculture products have neutral pH and *Salmonella* may be protected in this food matrix. The outcome may be affected by the amount of food ingested, the fat content and buffering capacity of the food, and the nature of contamination. In one outbreak linked to the consumption of scallop with egg yolk, 6.30 log cells resulted in a 56% attack rate. In fatty foods (eg chocolates, cheddar cheese) low infective dose may be observed and some aquacultured fish (eg salmon, catfish) may have high fat content, but there is no available data on outbreaks associated with these fish. Increased attack rates have been associated with ingestion of Salmonella between meals and it has been postulated that pyloric barrier may fail at this time and chocolates and ice creams may be consumed between meals (Mossel and Oei, 1975). Distribution of bacteria in food may also affect the outcome and due to the nature of bacteria to grow in colonies, agglomeration of cells may occur in foods and cells in inner layers of this might be protected (FAO/WHO, 2002).

There is very little quantitative data on *Salmonella* in fish and fishery products. One study of imported fish in Japan showed a level (MPN)of about 30-40 cells/100g (Asai et al, 2007). Considering that $>10^5$ cells are required to cause infection, it can be suggested that multiplication in fish would be necessary before the food is consumed. *Salmonella* is a mesophilic organism and the growth rate of this organism is markedly reduced at temperatures <15°C while the growth of most strains is prevented at <7°C (ICMSF, 1996). Most studies on minimum growth temperature have been done with beef, chicken or eggs using serovars like Typhimurium or Enteritidis common in these foods. However, these are not common serotypes in seafoods. In raw seafoods containing a variety of bacteria, *Salmonella*, if present has to compete with other flora for growth. *S.* Heidelberg had a generation time of 28h and 31h in the fish English sole and sterile crab respectively at 8°C (ICMSF, 1996). In cooked crab inoculated with *Salmonella* and stored at 8-11°C under modified atmospheres containing low levels of CO_2 (20-50%) proliferation of *Salmonella* has been reported (Ingham et al., 1990). *Salmonella* have ability to proliferate at pH values ranging from 3.8 to 9.5 with optimum being 7.0-7.5 (ICMSF, 1996). Growth of *Salmonella* is generally inhibited at 3-4% NaCl, but salt tolerance increases with increasing temperature in the range 10-30°C (D'Aoust

and Maurer, 2007) and minimum water activity for growth is 0.94 (ICMSF, 1996). Though the resistance of *Salmonella* to drying varies, this organism may survive for months or even years in dried products and has been frequently isolated from fish meal, meat and bone meal, maize and soy products (Lunestad et al., 2007). Decrease in *Salmonella* numbers occur during freezing and frozen storage, but this process does not guarantee elimination of salmonellae in foods (ICMSF, 1996). *Salmonella* are heat sensitive and D-values are influenced by the water activity, nature of the solutes and pH of the suspending medium (ICMSF, 1996). Typical D-values reported for *Salmonella* are 0.176min in chicken at 70°C, 0.36min in ground beef at 63°C (FAO/WHO, 2002). Some strains of *Salmonella* like S. Senftenberg 775W my show higher heat resistance (ICMSF, 1996). Interestingly, S. Senftenberg is the serovar often isolated from fish feed (Lunestad et al., 2007).

Public Health Outcomes

Manifestations of disease

Symptoms associated with Ingestion of foods contaminated with *Salmonella* could result in a variety of adverse health effects ranging from predominantly gastroenteritis to systemic infections which are much rarer. Gastroenteritis is the most common symptom associated with food borne non typhoidal salmonellosis. Acute illness is characterized by nausea, vomiting, diarrhoea, abdominal cramps, headache and fever. The incubation period for acute gastroenteritis is generally between 12 to 72 hours and illness may lasts between 2 to 7 days. The available data measuring illness as the endpoint suggests that no response is observed until a dose of 10^6 is reached (Coleman and Marks, 1998). Severe dehydration due to diarrhea can on occasion require medical intervention through the administration intravenous fluids and antibiotic treatment. However, on occasion this pathogen may come septic after entering the blood stream from the intestine and required intense medical intervention. Death is rare if patient is promptly hydrated and provided antibiotic treatment.

Rationale for the biological end points modeled

Acute gastroenteritis illness was modeled as the endpoint in this risk assessment associated with salmonella infection in countries exporting or importing seafood products. Nine studies have been published of experimentally induced salmonellosis, conducted between 1936 and 1970 using a variety of serotypes and strains (Table 2). However, some of these studies were deemed to be unsuitable and were not used in further analysis to derive conclusions about the pathogenicity of *Salmonella* in general in humans. Severe illness resulting from salmonellosis can be exacerbated by antibiotic resistant strains of *Salmonella* and may be further complicated by the effects of other underlying illnesses.

Hazard Characterization

Outbreaks associated fish and fishery products

Among food borne outbreaks reported in International literature between 1988 and 2007, for which a source could be identified (n= 4093), 46.9% was due to *Salmonella* (Greig and Ravel, 2009). In an analysis of globally reported food borne illnesses due to *Salmonella*, seafood accounted for 1.7% compared to 14% associated with eggs (values calculated from Table 3 of Greig and Ravel, 2009). Non-Typhi salmonellae cause an estimated 1.4 million illnesses in the United States each year, resulting in an estimated 15,000 hospitalizations and 400 deaths (Voetsch et al., 2004).

Outbreaks involving seafood has been reported from some countries. In Japan, *S.* Champaign was involved in 330 cases in children, who consumed cuttlefish that had been left to thaw at room temperature for 30h and then boiled for a short period (Ogawa et al., 1991). Contaminated well water of a squid processing plant in Japan was found to be the source of *Salmonella* that affected more than 400 people in 1999 and during the same year, cuttlefish snack contaminated with *S.* Chester was involved in an outbreak that affected more than 1500 people (D'Aoust and Maurer, 2007). *S.* Livingstone was the cause of an outbreak that occurred in Norway and Sweden in 2001 in which fish gratin manufactured in Sweden was implicated and the egg powder ingredient in fish gratin was suspected to be the source (D'Aoust and Maurer, 2007). One outbreak in which 16 people became ill after a reception in a hotel in UK in 1981 was attributed to frozen prawns (PHLSC, 1983). Though the implicated food has not been tested, only those who ate prawns were affected and S. Bareilly and S. Hindmarsh was isolated from the patients. It is not clear whether the prawns were prepared with any other ingredients, which could be a source of *Salmonella*.

Dose-response relationship

There are number of human feeding trials performed using six different serotypes. There were usually no responses at doses less than 10^6. However, outbreak investigations show that lower number of cells can cause infection depending upon the food matrix. There is no data with sea food matrix alone but in an outbreak of S Enteritidis associated with scallop and egg yolk, a 56% attack rate was observed at a dose of 6.3 log CFU. More information on the outbreaks, attack rate and doses (please see Table 3.14 of the document) involved is available in the document of Risk Assessment of *Salmonella* in eggs and broiler chicken (FAO/WHO, 2002).

Hazard Characterization

Table 1. Dominant Salmonella Serotypes Associated with Human Illness, and Seafood/Aquaculture Environment

Human Illness Associated Global Rank[a] 2002	Seafood Associated Rank Occurrence[b] 1990- 1998	Aquaculture Environment[c] 2001- 2003
Enteritidis (1)	Weltevreden (1)	Weltevreden
Typhimurium (2)	Senftenberg (2)	Paratyphi-B
Newport (3)	Lexington (3)	Senftenberg
Heidelberg (4)	Paratyphi-B (4)	Houten
Infantis (5)	Enteritidis (5)	Abaetetuba
Hadar (6)	Newport (6)	Derby
Virchow (7)	Thompson (7)	Aberdeen
Javiana (8)	Lanka (8)	Javiana
Saintpaul (9)	Virchow (9)	Hvittingfoos
Montevideo (10)	Hvittingfoss (10)	Give
	Typhimurium	Newport
	Derby(14)	

[a]Paratyphi B- #16; Weltevreden #20

[b]Typhimurium- #12; Derby #14

[c]not rank ordered

Refs

Galanis et al. 2006;

Kumar et al., 2009

Heinitz et al. 2000;

Hatha et al 2003;

Koonse et al. 2005;

Norhana et al. 2010

Reilly et al 1992.

Table 2. Human feeding trials using *Salmonella*

No.	Serotype(s)	Strain(s)	References
1	S. Typhimurium		Hormaeche, Peluffo and Aleppo, 1936
2	S. Anatum		Varela and Olarte, 1942
3	S. Meleagridis	I, II & III	McCullough and Eisele, 1951a
	S. Anatum	I, II & III	McCullough and Eisele, 1951a
4	S. Newport		McCullough and Eisele, 1951c
	S. Derby		McCullough and Eisele, 1951c
	S. Bareilly		McCullough and Eisele, 1951c
5	S. Pullorum	I, II, III & IV	McCullough and Eisele, 1951d
6	S. Typhi		Sprinz et al., 1966
7	S. Sofia S. Bovismorbificans		Mackenzie and Livingstone, 1968
8	S. Typhi	Quailes, Zermatt, Ty2V, 0-901	Hornick et al., 1970
9	S. Typhi	Quailes	Woodward, 1980