

## *Salmonella* spp.

Maha Hajmeer

### **Historical aspects**

*Salmonella* is not a recently discovered microorganism. *Salmonella typhi* was discovered in 1874, and isolated in pure culture in 1884.

The first laboratory-confirmed outbreak of salmonellosis was reported by Gaertner in 1888. This was caused by non-typhoid *Salmonella*, called *Bacillus enteritidis* by Gaertner. A total of 57 people got gastroenteritis from eating beef from a sick cow.

An outbreak of salmonellosis (caused by *B. enteritidis*) occurred in 1896 and was reported by van Ermengem — famous for discovery of *Clostridium botulinum*. Several workers (including a veterinary meat inspector, some plant workers, and the plant director) got sick from eating sausage. The pathogen was isolated from diseased individuals.

Salmon and Smith isolated a similar organism from pigs with hog cholera and described it in detail. It was called *Salmonella cholera-suis*; thus the genus *Salmonella* was created.

The bacterium can be pathogenic in man and animals. Investigations showed that the ecological habitat for this microorganism is the intestinal tract of warm- and cold-blooded animals. This enteric pathogen may colonize the intestines of individuals and animals, some might be shedders and asymptomatic.

The effect of *Salmonella* on animals can be spectacular.

- *S. enteritidis* isolated from a sick child was found to kill up to 50% of wild rat populations. “Ratin” was used as a rodenticide in Europe until 1945.
- In Greece, scientists used the mouse typhoid (*S. Typhimurium*) to reduce large mouse populations.

In 1943 in Sweden, it was found that 26% of wild rats were carriers of *S. enteritidis* and/or *S. Typhimurium*. These creatures still seem closely associated also in the US.

**Classification of *Salmonella***

Family Enterobacteriaceae

Genus *Salmonella*

Three Species:

1. *Salmonella typhi*
2. *Salmonella cholerae suis*
3. *Salmonella enterica*

*Salmonella enterica* > 2,400 Serotypes

*Salmonella enterica* var. *typhimurium* → now called *Salmonella typhimurium* or Typhimurium

*Salmonella enterica* var. *enteritidis* → now called *Salmonella enteritidis* or Enteritidis

Serotyping based on three cell-surface antigens:

1. The O, or cell-wall (somatic) antigen,
2. The H, or flagellar antigen, and
3. The Vi (outer polysaccharide layer) antigen

Most salmonellae are named after the place where they were first found. For example, *Salmonella cubana* and *Salmonella braenderup*.

**Characteristics of *Salmonella***

Gram negative, aerobic or facultative anaerobic, motile or non-motile, non-sporeforming rods. Catalase positive and oxidase negative. Produces acid, sometimes, and gas from glucose fermentation, seldom lactose. Reduces nitrate to nitrite. Grows readily on very simple media. Has 90% DNA homology with *E. coli*. Little or no correlation between serotype and clinical signs and symptoms.

**Estimated salmonellosis in the US**

1,341,873 cases per year, 9.7% of total

15,608 hospitalized, 25.6% of total

55 deaths, 30.6% of total

**Reported**

<b>YEAR</b>	<b>% OF OUTBREAKS</b>	<b>% OF CASES</b>	<b>% OF DEATHS</b>
1993	13.9	40.8	11.1
1994	10.7	17.6	33.3
1995	14.3	47.5	81.8
1996	14.5	55.1	50
1997	11.9	14.5	0

**Foods involved in outbreaks**

<b>Food Vehicle</b>	<b>Number of Outbreaks</b>
Milk	2
Cheese	1
Eggs	14
Ice cream	6
Baked goods	10
Fruits and vegetables	9
Beef	14
Ham	1
Pork	3
Chicken	5
Turkey	6
Multiple/Other	61

**Prevalence in supermarket foods**

<b>Food Item</b>	<b>Percent Positive</b>
Beef	0 - 2.6
Veal	4.1
Pork	0 - 18
Pork Products	3 - 20
Turkey	70
Turkey Sausage	100
Chicken	0 - 100
Shellfish	4 - 33

**Factors contributing to salmonellosis**

<b>Factors affecting food</b>	<b>% of outbreaks in</b>	
	<b>USA</b>	<b>England</b>
<b>Prepared &gt;12 hours before</b>	23	49
<b>Stored at room temperature</b>	--	29
<b>Inadequate cooling</b>	43	18
<b>Inadequate re-heating</b>	12	12
<b>Improper hot-holding</b>	--	13
<b>Under cooking</b>	24	23
<b>Inadequate thawing</b>	--	11
<b>Cross contamination</b>	20	14
<b>Improper cleaning</b>	--	13
<b>Contamination of raw material</b>	31	--
<b>Food eaten raw</b>	--	9
<b>Contamination of processed food</b>	--	27
<b>Others</b>	14	8

**Salmonella infections**

- Most *Salmonella* spp. infect both man and animals, and some are host-adapted or host-specific.
- *S. typhi* \_ Man
- *S. pullorum* and *S. gallinarum* \_ Poultry
- Some have host preferences: *S. dublin* in Cattle

**Salmonella syndromes**

There are three different syndromes of *Salmonella*:

1. Typhoid fever → most severe of the three syndromes
  - caused by *S. typhi*
  - human specific
  - imply human fecal contamination of food or water
  - fortunately this type of human salmonellosis is <2.5% in the U.S.

With this syndrome, normally the organism is ingested → gets to small intestine → penetrates epithelial cells of the villi → ultimately gets into the lymphatic system → phagocytosed by macrophages → multiplies within macrophages → spills out into blood → gets to liver, spleen, gall bladder, and other organs → causes systemic infections.

2. Enteric fever → *S. paratyphi* A
  - *S. paratyphi* B (renamed *S. schottmuelleri*)
  - *S. paratyphi* C (renamed *S. hirshfeldii*)
3. Gastroenteritis syndrome → caused by all other types of *Salmonella*
  - > 2400 serotypes of *Salmonella*
  - 150 of them have been associated with human disease

**Infection in man \_ typhoid fever**

- Systemic febrile illness caused by *S. typhi*
- Food and water contaminated with human feces
- Incubation 1 week to 1 month
- *S. typhi* enters body once ingested through M cells of lymphoid tissue in intestinal tract
- Seldom diarrhea but ulceration of intestine
- Multiplies in liver and spleen
- Causes high fever, chills, convulsions, delirium
- Toxins are not well defined
- Case fatality rate about 10%
- May persist in gall bladder for years
- "Typhoid Mary" \_ worked for families (house servant), rather than in restaurants

### **Overall symptoms of *Salmonella* infection**

- Nausea and vomiting for a few hours followed by abdominal pain and diarrhea, sometimes fever. Symptoms are caused by invasion of intestinal mucosal cells, and the organism grows inside fixed macrophages. Immunocompromised may get systemic infections. Other associated illnesses include arthritis / Reuters syndrome (3 – 4 weeks).
- Infective dose maybe as few as 15 – 20 cells.
- Incubation period of the microorganism is 6 – 48 hours.
- Signs and symptoms may appear as soon as 6 hours and last up to a week.
- Severity varies from person to person.
- Duration of illness is 1 – 2 days, but it may last longer.
- Infected persons will shed salmonellae for 1 – 3 months, and a few (1 – 3%) may shed for a year.
- Most cases in children are in individuals below 10 years of age.

### **Infection / colonization of animals**

- Almost any vertebrate animal can become infected with salmonellae.
- Mostly the infection does not cause clinical disease, and probably it is more appropriate to call it colonization.
- Animals can become carriers, but most stop shedding after a few weeks.
- Latent infections may “Flare Up.”
- Animals \_ like man \_ may become infected from water or feed, but a contaminated environment is the most important direct source.
- Lack of sanitary precautions among animals might be a big contributing factor to animal colonization and infection with *Salmonella*.

### **Infective dose in man and animals**

- The young are more susceptible than adults.
- Day old chicks 1 – 5
- Four week old 100 – 1,000
- Adult hens 10,000 or more
- 50 – 60 lb pigs 10,000 or more
- Infants 100 or less
- Adult human male 100,000 or more

Some reject the notion of a minimum infective dose and claim any number of *Salmonella* can cause infection, the probability of which varies with the numbers. Issues such as virulence of *Salmonella* strain, individual tolerances and susceptibility, and stomach content need to be considered.

Foods rich in lipids, such as chocolate or ice-cream, protect *Salmonella* against stomach acid, and it takes only a few cells to produce infection.

**Reservoirs**

- A reservoir is the “place” an organism depends on for its perpetuation.
- The reservoir for salmonellae is the intestinal tract of vertebrates.
- Under the right circumstances it can multiply temporarily in the environment including foods
- The prevalence of *Salmonella* in humans in USA is about 0.1%
- The prevalence in animals is generally higher, between 0 and 30%

**Transmission**

- Fecal material, since the primary reservoir of *Salmonella* is fecal material.
- Person-to-person contact — note that transmission among humans, for example via food, can not be excluded. Some humans as well as animals might be shedders.
- Contact with animals (e.g., pets). The Centers for Disease Control and Prevention (CDC) estimated that about 280,000 cases of *Salmonella* annually were linked to pet turtles.
- Aerosols — although very unlikely.

**Growth and survival of *Salmonella*****Growth:**

- *Salmonella* is not a fastidious microorganism. It can grow in simple glucose-salts medium, and it can grow more rapidly in highly supplemented media.
- Growth pH values are between 4 and 9, but some can grow at pH of 3.7.
- Water activity is between 0.96 and 0.999. The microorganism dies rapidly at 0.7 – 0.8, and more slowly at lower values.
- Oxidation-reduction (OR) potential: This has little effect on growth or survival of microorganism.

**Survival:**

- *Salmonella* can be killed by repeated freezing and thawing cycles of food, but this might have an adverse effect of food quality.
- Heating can kill *Salmonella*. The growth temperature of this microorganism is between 5 – 45°C with an optimum growth temperature of 35 – 37°C. Some strains are more resistant to heat than others.
- For example, *S. senftenberg* 775W is ~10 – 20 times more resistant to heat than an average strain of *Salmonella*, but it is rare.
- At 60°C the decimal reduction time (D value) is between 0.4 and 0.6 min.  
One D value equals 90% kill  
Two D values equal 99% kill, and so on
- The z-value (change in temp. needed to change D value by a factor of 10) is 4 – 5°C
- Thus D value at 55°C is 4 – 6 min
- *Salmonella* is more resistant in egg yolk than in white
- Also, it is highly resistant in dry foods and foods rich in fat.
- In milk chocolate with less than 2% moisture the D-value is 222 min (3.7 h) at 80°C

**Competition against other microorganisms**

- *Salmonella* has short lag phase and can grow rapidly.
- Intestinal flora of adult hens is inhibitory: “competitive exclusion”
- Five percent (5%) of *Salmonella* strains produce bacteriocins against *E. coli*, *Shigella* or *Salmonella*

**Detection of *Salmonella***

- Non-selective pre-enrichment to repair cell damage, rehydration, and dilution of toxic substances  
Lactose broth  
Buffered peptone  
Etc.
- Selective enrichment to inhibit other bacteria  
Tetrathionate  
Selenite cystine  
Rappaport-Vasiliadis (Malachite green)
- Differential selective agar media to select and identify *Salmonella*  
Brilliant green novobiocin  
XLT4  
Bismuth sulfite
- Biochemical confirmation
- Serology
- Rapid Method  
Immunoassay  
Gene probes, PCR, and electrical measurements conductance

**Selected references:**

Bryan, F. L., Fanelli, M.J., and Riemann, H. 1979. *Salmonella infections*. pp. 73-130. In H. Riemann and F.L. Bryan (eds) 1979. Food-borne Infections and Intoxications, 2d ed, Academic Press, New York.

Gray, J.T., and Fedorka-Cray, P.J. 2002. *Salmonella*. In Foodborne Diseases. 2<sup>nd</sup> Ed. Cliver, D.O. and Riemann, H. (Eds.) Academic Press, New York, NY. Chapter 3. pp. 55-68.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P., and Tauxe, R. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5(5): 607-625.

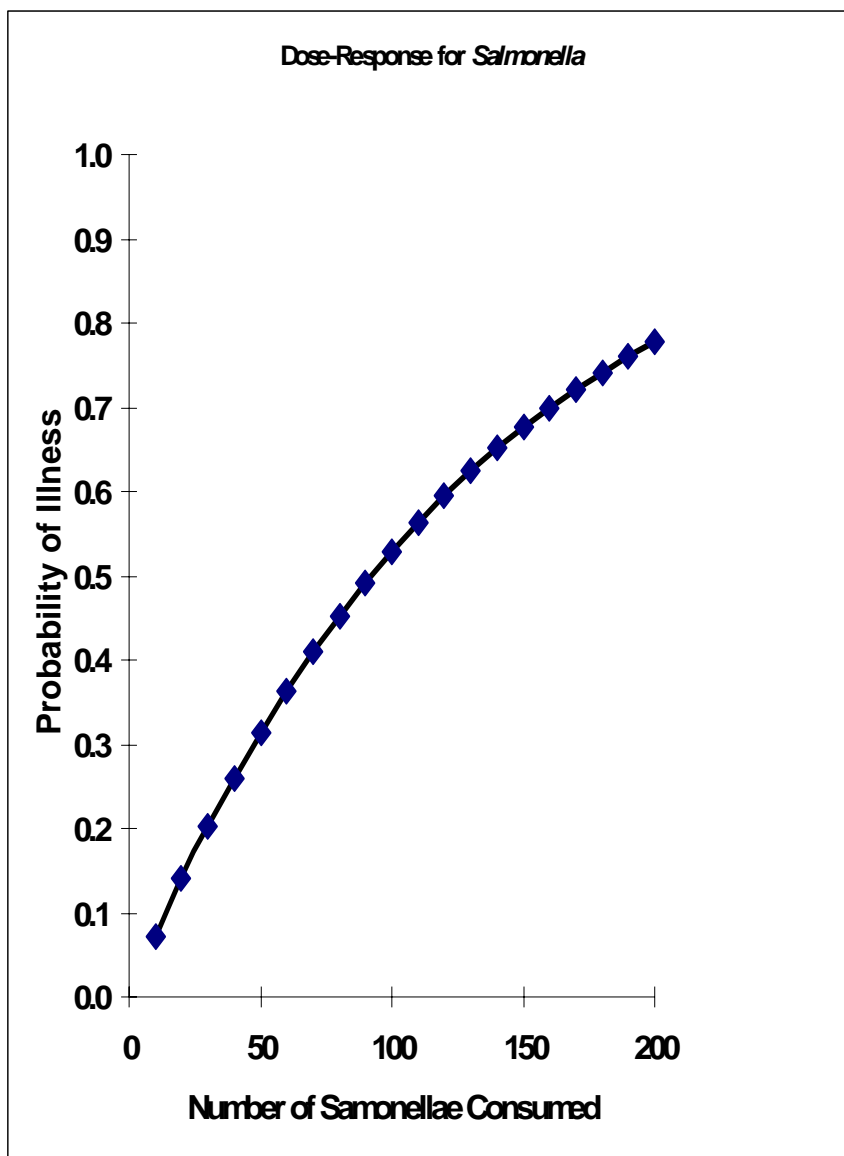
Molbak, K., Olsen, J.E., and Wegener, H.C. 2006. *Salmonella infections*. pp. 56-136. In H. Riemann and D.O. Cliver (eds). Foodborne Infections and Intoxications, 3rd ed, Academic Press, New York.



**Fig. 1 Infectious dose of Salmonellae for humans.**

$P = 1 - e^{-rN}$ , where  $P$ =probability of infection,  $r=0.00752$  (Salmon), and  $N$ =numbers of salmonellae consumed.

N	P
10	0.0724
20	0.1396
30	0.2020
40	0.2598
50	0.3134
60	0.3631
70	0.4093
80	0.4521
90	0.4918
100	0.5286
110	0.5627
120	0.5944
130	0.6238
140	0.6510
150	0.6763
160	0.6998
170	0.7215
180	0.7417
190	0.7604
200	0.7778



**Fig. 2** Survival of *S. enteritidis* in chicken manure at different water activities and a temperature of 20°C.

Hours	Water activity	
	0.97-1.00	0.75-0.80
2	6.1	5.8
4	6.5	4.8
6	6.4	3.9
8	6.5	3.6

