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Salmonella spp.

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1 Introduction, history and contemporary problems

1.1 Introduction

Infections with *Salmonella* are a major cause of bacterial foodborne diarrhea in humans worldwide. These bacterial pathogens are crucial zoonotic agents in the veterinary as well as medical field. Sporadic cases of human infections due to contact with household animals shedding *Salmonella* have repeatedly been reported; however, prevalence of *Salmonella* spp was 0% for all samples from 94 wild turtles.

1.2 History

Water and milk were found to be vehicles of the etiologic agent of enteric fever by epidemiological evidence several years before the agent itself was identified in 1874. The organism, now named *Salmonella enterica* serotype Typhi, was discovered in 1880.
While *S*. Typhi became an enormous problem in the US in the early industrial era, the disease burden associated with non-typhoid *Salmonella* was low before World War II.
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 with *Salmonella* also in the US. There are limited historical data on the prevalence of *Salmonella* in healthy animals, carcasses or meat.
Improvements in sanitation nearly eliminated *S*. Typhi as a cause of indigenous infections in the US and other developed countries. Decades later, non-typhoid *Salmonella* infections began to increase in importance – a trend that may have peaked near 1990.
In 2006, 2496 farm samples were collected quarterly from 18 different farms across five states (Alabama, California, North Carolina, Tennessee, and Washington) over a 24-month period. *Salmonella* isolates were recovered from 4.7% of all samples.

1.3 Contemporary problems

Non-typhoid *Salmonella* infections in humans continue to be a major problem, in terms of both morbidity and economic costs. Most human *Salmonella* outbreaks are associated with the consumption of contaminated products of animal origin, although non-foodborne *Salmonella* infection in humans may be transmitted during contact with animals, contaminated water, or the environment.
The widespread distribution of food is a global challenge in *Salmonella* control. With increasing travel and global trade, outbreaks involving widely scattered cases are occurring more frequently.
Recent studies in England, Holland and US suggest that for every reported *salmonella* infection, between 3.8 and 38 persons actually fell ill; 1.4 million infections on non-typhoidal *Salmonella* is the resulting incidence estimate..

S. Enteritidis, in 1995, was the most frequent isolated serotype in 35 countries. It may colonize the ovaries of hens and causes vertical transmission to eggs. Food-producing animals are an important source of human non-typhoid *Salmonella* infection in Vietnam.

2. 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.
Family Enterobacteriaceae
Genus *Salmonella*
Two Species:
1. *Salmonella enterica*
2. *Salmonella bongori*
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

3. *Salmonella* infection in man and animals

Most *Salmonella* serotypes infect both man and animals, and some are host-adapted or host-specific.
S. Typh*i* — Man
S. Pullorum and *S.* Gallinarum — Poultry
Some have host preferences: *S.* Dublin in Cattle
The incidence of non-typhoid *Salmonella* is usually highest in infants and young children, and usually the incidence in boysis higher than in girls.
Most *Salmonella* infections in the northern hemisphere are around August and in the south around March.
Comparison of reports of CDC between 1993 and 2003 shows about 100% more fatality in *Salmonella* outbreaks regardless of 40% decrease in outbreaks.
Salmonella has a very important role in foodborne diseases:

(i) *Salmonella* is among the most common bacterial causes of foodborne illness.
(ii) It is commonly present in many mammal and bird species, including all of the meat and poultry product classes produced under federal inspection, at frequencies that permit changes to be detected and monitored.
(iii) There are methodologies available to recover *Salmonella* from a variety of meat and poultry products.

(iv) Intervention strategies aimed at reducing contamination of raw products with feces and other sources of *Salmonella* will likely be effective against other foodborne pathogens

Among non-typhoid *Salmonella* isolates from humans and animal origin from feces, carcasses and meat in Vietnam, the most prevalent serovar was. S. Typhimurium.

3.1 *Salmonella* syndromes

There are three different syndromes of *Salmonella*:

1. Typhoid fever — most severe of the three syndromes
   - caused by *S.* Typhi
   - human specific
   - simply human fecal contamination of food or water
   - Fortunately, this type of human salmonellosis is just 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

3.2 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

3.3 Infective dose in man and animals

- Day old chicks 1 – 5 cells
- 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, ice-cream, or *peanut butter*, protect *Salmonella* against stomach acid, and it takes only a few cells to produce infection.

10^5 organisms of *S*. Typhi resulted in disease; however in an outbreak of *S*. Typhimurium infection from chocolate, ≤10 *S*. Typhimurium per 100 g of chocolate was found.

3.4 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 the USA is about 0.1%.
- The prevalence in animals is generally higher, between 0 and 30%.

3.5 Transmission

- Fecal material, since the primary reservoir of *Salmonella* is fecal material. The *Salmonella* infection is acquired by thefecal-oral route, although swallowing contaminated aerosol may cause infection.
- Raw or undercooked eggs, poultry, meat and unpasteurized milk, cross contamination of other foods during processing and recently, uncooked vegetables, fruits and direct or indirect contact with animals.
- Person-to-person contacts — 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.

4. Growth and survival of *Salmonella*

4.1 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.

- Optimal 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.
- The growth temperature range of this microorganism is between 5 – 45°C with an optimum growth temperature of 35 – 37°C.

4.2 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*. 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 heat-resistant in egg yolk than in white.
• Also, it is highly heat-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.

4.3 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*

4.4 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 Methods
Immunoassay
Gene probes, PCR, and electrical measurements conductance

5. Control of *Salmonella* in food and feed

5.1 Poultry products

5.1.1 Pre harvest control
Poultry products could be contaminated at several stages during slaughter: evisceration, cutting, defeathering, cross contamination on surfaces, chilling in a water bath reduces the load of *Salmonella*. However, this may enable cross contamination from contaminated to uncontaminated products. Correlation between levels of *Salmonella* in broiler flocks and carcasses after slaughter has been documented in some studies.
Prevention of bird flock contamination based on the Good Manufacturing Practices and Good Agricultural Practices, in addition to vaccination, control of feed stuff contamination, are most useful ways to prevent contamination of bird flocks before slaughter.

5.1.2 Post harvest control
Control of scalding temperature between 50°C and 60°C with counter-current or spray scalding
Control of defeathering, evisceration, system hygiene
Improvement of rinse chill system.

5.2 Shell eggs

5.2.1 Pre harvest control
Culling of infected birds and with *Salmonella*-free birds- note in the birds we can not see clinical signs of salmonellosis as in humans.
Vaccination
Management control

5.2.2 Post harvest control
Heat, Irradiation,
Whole egg pasteurization in 60.0°C for 3.5 minutes—expensive
Irradiation (3 kGy)

5.3 Pigs

In a recent study in US, samples were collected on farms (feces) and at slaughter (distal colonic content, cecal content and ileocolic lymph nodes). The mean individual pig prevalence was 5% for feces, 4% for distal colonic content, 15% for ileocolic lymph nodes, and 17% for cecal contents.
Serological surveillance of pig herds and slaughter of animals based on the serological surveillance result would be the recommended pre harvest and post harvest control in this regard.

5.4 Cattle, beef, and dairy products

Serological surveillance of cattle, establishing S*almonella*-free herds control and management of surface water and pastures after application of manure, slurry or sludge, effective rodent control program, control and prevention of wet areas, personnel hygiene, would be the recommended pre harvest controls in this regard.
Milk pasteurization and sufficient cooking temperature of beef would be the post harvest recommendations. In a study in Turkey, Tulum cheese, produced from raw milk *Salmonella* was found in 2.4% of samples.

5.5 New food vehicles

Contamination of new food vehicles typically occurs early in the production process. These foods have fewer barriers to microbial growth. *Salmonella* presence in fresh fruits and vegetables were investigated in a study by CDC. Contamination may occur during production and harvest, initial processing and packaging, distribution and final processing. Contaminated water seems a particular source of the contamination and chlorination of water could be a critical issue in this regard.

5.6 Feed

In one study in the Netherlands, 10% of poultry feeds and feed components were contaminated with *Salmonella*, and in another study in the US, 2.8% of feed and feed ingredients and 46.7% of farms were contaminated with *Salmonella.*
Pelleting temperature (82°C for 15 second) and Good Agricultural Practices would be effective ways to control salmonellosis in animal feed. Large outbreaks of canine salmonellosis occurrred in military watch dogs due to *Salmonella* Montevideo and/or *Salmonella* Give. This demonstrates not only that those *Salmonella* infections in dogs occur without clinical symptoms but also that large outbreaks occur after feeding dehydrated dog foods.

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