Non Typhoidal *Salmonella* in food products

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Abstract

Food-Borne Diseases (FDB) include a large number of diseases resulting from the ingestion of food contaminated with microorganism's toxins or other chemicals. One of the most common FBD is Salmonellosis, a zoonosis caused by *Salmonella*, considered as one of the four leading causes of zoonotic diseases in the world. The present review aims to collect and update information about *Salmonella* and its prevalence in food products worldwide based on published research studying this pathogenic organism in addition to a general view of regulations implemented by the World Health Organizations and recommendations guidelines adapted by food industries. After describing *Salmonella* and its different bacterial, biochemical and environmental characteristics, several research have been analyzed to determine prevalence of *Salmonella* in different countries to determine the most important sources of contamination of food products. The first source of contamination found is poultry and raw egg products due to ease of their contamination by *Salmonella*, while contamination of other food products was variable. Severe effects of *Salmonella* prove the importance of having regulations to limit the negative consequences of the emergence of *Salmonella*.

Keywords: *Salmonella*, Human Salmonellosis, Prevalence, food product

INTRODUCTION

Food-borne diseases (FBD) include a large number of diseases resulting from the ingestion of contaminated food by microorganisms toxins or other chemicals. Moreover, food contamination can occur at any stage, such as production, transportation and at the consumer level.

FBD are a worldwide growing public health problem and according to the WHO's first global estimates, every year nearly 600 million people, or almost 1 in 10 people worldwide, become ill after consuming contaminated food. Thus, diseases resulting from this consumption are considered one of the top 10 causes of death in the world with more than 422,000 deaths per year, of which 125,000 (30%) are children under 5 years who are more exposed to these types of diseases (WHO, 2015).

One of the most common FBD is Salmonellosis, a zoonosis caused by *Salmonella enterobacterium* considered as one of the four leading causes of zoonotic enteric diseases in the world with over 57,000 deaths and 153 million cases of gastroenteritis per year (CDC, 2020).

Salmonellosis poses a public health problem in all the regions of the world, in either high- or low-income countries. In France the National Reference Center for *Escherichia coli*, *Shigella* and *Salmonella* (NRC), estimates about 307 cases of salmonellosis per 100,000 inhabitants per year. In Canada, from 2009 to 2013, 6,500 cases of salmonellosis on average were reported (PHAC, 2016), in the countries of the European Union 91,857 cases were reported each year (AFSA, 2019) and 1,35 million infections were reported in the United States (CDC, 2020). However, the risk of these infections in low- and middle-income countries remains more severe, where it is associated with poor hygiene, food preparation and conservation conditions, and a lack of food safety legislation and its enforcement. In Africa, 80.3 million cases of salmonellosis are detected each year (Shannon *et al.*, 2010).

In Morocco, collective food toxi-infections (TIAC) constitute a public health emergency because of the virulence and pathogenicity of the bacteria involved and their morbidity potential if the source of infection is not identified or controlled. The increase in TIACs in recent years is largely due to the emergence of certain bacteria, in particular *Salmonella*. In 2006, 1664 TIAC cases were recorded, and only 121 were confirmed in laboratories, of which 50.4% (61 cases) were Salmonellosis.

TAXONOMIC STUDY

Classification

Salmonella belongs to the proteobacteria genus, which contains many pathogenic bacteria. The genus *Salmonella* is divided into two species: *enterica* and *bongori.*

The enterica species are divided into 6 subspecies:

S. enterica I or subspecies *enterica*.
S. enterica II or subspecies *salamae.*
S. enterica IIIa or subspecies *arizonae*.
S. enterica IIIb or subspecies *diarizonae*.
S. enterica IV or subspecies *houtenae*.
S. enterica VI or subspecies *indica*.

In these 2 species, more than 2,500 different serovars have been identified so far.

BACTERIOLOGICAL STUDY

Morphology

Salmonella are straight, non-spore-forming, non-capsulated sticks measuring approximately 0.7-1.5 µm in diameter for 2 to 5 µm in length (Percival and Williams, 2014).

They are usually flagellated in a periodical and mobile way, although non-mobile mutants of natural origin are sometimes encountered; *S. pullorum* and *S. gallinarum* are typically non-mobile (Gast and Porter, 2019).

Biochemical and cultural characteristics

The main biochemical characteristics of *Salmonella* are:

- Oxidase negative,
- Catalase positive,
- Urease negative,
- Lack of indole production,
- Glucose fermentation with gas production,
- No production of hydrogen sulfide,
- Nitrate reductase positive,
- Lactose negative,
- Lysine decarboxylase positive,
- Tryptophan deaminase (TDA) negative.

Salmonella multiply between 7°C and 45°C with an optimum at 35°C / 37°C and at pH of 4.1 to 9.0 with an optimum of 7 to 7.5 (ANSES, 2011).

Salmonella are an optional aerobic bacteria, they grow on an ordinary medium and a selective medium.

The diameter of *Salmonella*'s typical colonies on agar medium are about 2-4 mm, round with smooth edges, slightly raised and shiny.

Antigenic structure

The separation of *Salmonella* into serotypes can be based on the structure of their surface:

- The O antigen is the outermost portion of the bacteria's surface covering, which has a lipopolysaccharide nature and causes the synthesis of agglutinating antibodies.
- The H antigen is a slender threadlike structure that is part of the flagella, with a protein nature, thermolabiles, and produce agglutinating antibodies.
- The Vi antigen, only exists in three serovars: *S. Typhi*, *S. Para* C, *S. Dublin*. Its presence can mask the antigen O, making the strain "O inagglutinable". This inhibition can be lifted by the 100°C strain warming, with Vi antigen being thermolabile.

Habitat

The main reservoir of *Salmonella* spp. consists of the gastrointestinal tract of mammals (pigs, cattle) and birds (domestic poultry) (ANSES, 2011).

The *enterica* subspecies is suitable for warm-blooded animals (rodents, poultry) and water.

Other subspecies are associated with cold-blooded animals (reptiles, turtles and batrachians).

Salmonella in animal fecal matter can contaminate pastures, soils and water. It can last for weeks in water and months or years in the earth (Gast and Porter, 2019).

Contamination

Salmonellosis is mainly occurred by the direct ingestion of food from contaminated animals consumed raw or undercooked (meat, eggs, raw egg products). Other foods, such as green vegetables contaminated with manure, which have been involved in the transmission, or indirectly through the ingestion of a safe food contaminated with other food or water. The part of transmission by contaminated food ingestion is estimated at 95% for non-typhoid *Salmonella* (WHO, 2018).

Contamination also comes from pets, cats, dogs, birds and reptiles, such as turtles.

Human-to-human transmission via fecal-oral transmission is also possible.

Survival outside host

The Serotype Choleraesuis can survive in wet swine feces and in dry swine feces for at least 3 and 13 months respectively (Gray and Fedorka-Cray, 2001).

Serotype Dublin have the ability to survive in feces spread on concrete, rubber and polyester for 6 years (Block, 2001).

Certain serotypes are capable of surviving on fingertips for 80 minutes, depending on the inoculum size (Pether and Gilbert, 1971), which explain the facility of their transfer to food by handling.

HUMAN SALMONELLOSIS

Symptoms

Salmonellosis is usually characterized by sudden onset of fever, abdominal pain, diarrhea, nausea and sometimes vomiting (Table 1).

The incubation period is generally from 6 to 48 hours after consumption of the contaminated food, and the disease lasts from 2 to 7 days (WHO, 2018).

Generally, symptoms of salmonellosis are mild and in most cases the recovery doesn't require any special treat-

Table 1: Characteristics of non-typhoid Salmonellosis

Incubation time	High-risk population	Main symptoms	Symptoms Duration	Length of contagious period	Complications	Asymptomatic form
6 to 48 hours	Young children Elderly Immuno-depressed	Fever 39-40 °C Abdominal pain Diarrhea Nausea Vomiting	2 to 7 days	Several days to several weeks. Sometimes several months: 1% of adult patients and 5% of children under 5 years may remain excretory for less than 12 months.	Bacteria in 3-10% of cases Dehydration Hospitalization rate (22.1%) Mortality rate (0.8%)	Yes

ment. Yet, in some cases, such as young children and the elderly, the dehydration associated to the disease can become severe and lead to a life-threatening prognosis.

Once the symptoms are gone, the bacteria can still be present in the feces and the contaminated person can still contagious for several weeks. About 1 percent of infected adults and 5 percent of children younger than five shed *Salmonella* in their stool for up to one year (Redbook, 2018).

Diagnosis

Stool test is the most effective method in cases of gastroenteritis. In newborns, young children, the elderly and immunocompromised, minor *Salmonella* (non-typhic) are likely to cross the intestinal barrier and cause typhoid septicemic syndrome with positive blood culture.

Sensitivity

Salmonella's resistance to antimicrobials and disinfectants has increased considerably over the years, due to their inappropriate usage

Antimicrobial sensitivity: *Salmonella* are susceptible to chloramphenicol, ciprofloxacin, amoxicillin, cotrimoxazole, trimethoprim-sulfamide, cephalosporins and norfloxacin.

Physical inactivation: *Salmonella* are sensitive to heat. Cooking for 5 to 6 minutes at 65°C is sufficient to reduce the risk of salmonellosis. The cold blocks the development of *Salmonella* but does not eliminate them. *Salmonella* spp. can also be disinfected with ozone (Block , 2001).

Disinfectant sensitivity: The most commonly used disinfectants contain aldehydes as active substance, or a combination of aldehydes and quaternary ammonium compounds.

Chlorine and peroxides are very good disinfectants but they are entirely inactivated by the slightest presence of organic matter and can't be used for soil or material disinfection due to the corrosive effect (Pierré, 2013).

SALMONELLA IN FOOD

The involved foods are mainly eggs and egg products made from raw or poorly heat treated eggs, dairy products (raw or low heat milk) and meat (cattle, pigs and poultry). However, the cases described in the literature refer to many other foods (plants, shellfish, etc.) (ANSES, 2011) (Table 2).

Poultry

Poultry represents the first reservoir of *Salmonella* (Gupta *et al.*, 1999), so multiple studies have analyzed the prevalence of *Salmonella* in poultry products mainly for the chicken industry; the results have shown different rates of contamination.

For chicken carcasses, contamination rates were 57% in Portugal, 22% in the United Kingdom, 45.9% in Vietnam, 13% in South Africa, 27% in Columbia and 42% in China.

The contamination rates for chicken meat were 16% in Ecuador, 33% in Wales, 31% in Russia and 36% in Spain were registered.

Table 2: Prevalence of *Salmonella* in food

	Product	Country	Number of samples	Positive samples	Reference
Poultry	Chicken carcasses	Portugal	300	171 (57%)	Machado and Bernardo., 1990
		United Kingdom	449	100 (22%)	Uyttendaele et al., 1998
		Vietnam	1000	459 (46%)	TA *et al.*, 2012
		South Africa	99	19 (19%)	Nierop, *et al.*, 2005
		Columbia	1003	270 (27%)	Donado-Godoy *et al.*, 2012
		China	1595	665 (42%)	Zhu *et al.*, 2014
	Turkey parts	United Kingdom	434	47 (11%)	Uyttendaele *et al.*, 1998
	Chopped turkey meat	Morocco	192	39 (20%)	Karraouan *et al.*, 2010
	Turkey carcase	Morocco	192	47 (24%)	El Allaoui *et al.*, 2013
	Flesh chickens	Ecuador	388	62 (16%)	Vinueza-Burgos *et al.*, 2016
	Chicken	Wales	300	99 (33%)	Harrison *et al.*, 2001
	Chicken	Russia	698	219,87 (31%)	Alali *et al.*, 2012
	Chicken	Spain	198	71 (36%)	Domínguez, *et al.*, 2002
Meat	Carcass guinea fowl	United Kingdom	81	17 (21%)	Uyttendaele *et al.*, 1998
	Raw beef	United Kingdom	1563	21 (2%)	Little *et al.*, 2008
	Raw beef	Malaysia	156	24 (15%)	Shafini *et al.*, 2017
	Raw pork meat	United Kingdom	1440	56 (4%)	Little *et al.*, 2008
	Pork	China	387	81 (21%)	Yan *et al.*, 2010
	Game and other meat	United Kingdom	51	1 (2%)	Little *et al.*, 2008
Various	Vegetables	Brazil	512	4 (1%)	Sant'Ana *et al.*, 2011
	Tulum cheese	Turkey	250	6 (2.4%)	Colak *et al.*, 2007
	Seafood	North china	20	96 (21%)	Yan *et al.*, 2010
Milk	Milk powder	United States	5714	9 (0,16%)	Hayman *et al.*, 2020
	Raw milk	Tanzania	75	28 (37%)	Lubote *et al.*, 2014

A study in Morocco has shown a large rate of contamination by *Salmonella* in broiler chicken in Meknes region, *Salmonella* was isolated from 24% of the farms examined.

Meat

Studies on the prevalence of *Salmonella* in red meat are fewer, yet researches have shown remarkable contamination rates; 21% in the United Kingdom and 24% in Malaysia.

Relative to pork meat, the National *Salmonella* Reference Center of Pasteur Institute and the The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) are warning about the increase in the contamination in pork meat by *Salmonella*, and studies confirm the increase in contamination rates in the main pork meat producing countries.

Raw eggs

The egg white is a complex biological fluid that plays a major role in the protection of eggs against microorganisms. Its natural components such as ovotransferrin and lysosyme protect against a large panel of bacteria due to their antibacterial effect.

Those proteins could act in synergy with other components of the egg white such as the natural alkaline pH, and the ambient temperature (GUYOT *et al.*, 2013). However, the contamination of the internal contents of chicken eggs may be due to bird ovary infection (Barnhart *et al.*,1991), whereas shell contamination may be due to excrement, food, insects or material handling, transport or storage.

Milk

Raw milk is not frequently contaminated with *Salmonella* and this contamination is most often due to external factors, the cleanliness of the animal, in particular the udders, the surrounding environment, the milkman, the milk harvesting equipment, and finally the storage and transport equipment.

However, pasteurized milk is usually free of *Salmonella* because it is removed during pasteurization (Brisabois *et al.*, 1997).

Water

Salmonella may be present in agricultural and domestic waste water, fresh water, including water intended for human consumption and groundwater, and in seawater (ISO 19250:2010).

Salmonella regulation

In the European Union, Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs, defined in its first chapter *Salmonella* as a criterion for the safety of foodstuffs which present a non conformity for products placed on the market during their shelf life (Absence in 25 g) on a sampling plan equal to 5.

In 2011, Regulation (EC) No 1086/2011 and amended Regulation (EC) No 2073/2005 consider *Salmonella typhimurium* and *Salmonella enteritidis* as other safety criteria for fresh poultry meat in order to reduce the prevalence of these two *Salmonella* serotypes in broilers in the Union, therefore systematic serotyping of colonies should be carried out when the presence of *Salmonella* spp. is detected.

In 2020, *Salmonella* is defined as a safety criterion for a new matrix. The European Commission regulated reptile meat, even though these types of meat are not widely consumed in the Union except for the parts that are edible such as the tail of the crocodile used for its high protein content and low fatty acid.

In the second chapter of the regulation, *Salmonella* is considered to be a process hygiene criterion for the different types of carcasses. Moreover, in 2011 the level of tolerance of the presence of *Salmonella* on poultry carcases on a sampling plan of 50 decreased from 7 to 5 samples.

Also, in Morocco, according to the latest decision of the Minister of Agriculture, Maritime Fisheries, Rural Development and Waters and Forestry and the Minister of Health n°293-19, *Salmonella* is considered as an indicator of the safety of certain food products and as an indicator of hygiene for poultry slaughter processes (Official Bulletin n°6796 of 18/07/2019, page 1686).

Food safety guidelines

In raw eggs products

Since eggs can harbour *Salmonella* on the surface, and the raw eggs products are generally consumed without any further cooking, it is necessary that they are prepared and handled correctly and safely.

According to guidelines, the handling of eggs and raw eggs products can be done by two different methods: acidification or pasteurization.

The acidification of raw eggs products should be at a pH less than 4.2 through the addition of acidic ingredients such as lemon or vinegar, in order to inhibit the growth of pathogenic bacteria, including *Salmonella* (NSW Food Authority, 2016). The pH must be checked by pH paper or pH meter to make sure it has reached the 4.2 pH limit, then the product must be refrigerated at or below 5°C.

Eggs can be pasteurized in shell using vacuum method, by putting eggs in a water bath at temperature of 57°C for at least 75 minutes (Baldwin, 2010), at this point the pasteurized eggs must be used immediately or cooled in a 50:50 ice-water bath and refrigerated at or below 5°C for 10 days.

Milk products

The fermentation of milk products like yogurt can lead to acidification. The fermented product presents naturally a low pH that inhibits the proliferation of *Salmonella*.

For cheese, the development of *Salmonella* during processing, refining and acidification is simulated according to the physico-chemical parameters of the product (temperature, pH, water activity (aw) and concentration of lactic acid in its non-dissociated form).

During salting, the growth of *Salmonella* is inhibited using salt content above 3-4%. However, few cheeses achieve such high salt levels.

During acidification, *Salmonella* appears to be relatively inhibited by lactic flora, when the lactic acid achieve concentrations above 1%. Lactic acid in its non-dissociated form acts as a bacteriostatic antibiotic especially on pathogenic bacteria such as *Salmonella*. Moreover, it is considered to be a more effective decontamination agent than acetic acid (Deumier, 2004).

Aquatic by products

The aquatic animal's by-products should be reduced to fragments in order to apply a thermal treatment, and decrease their pH using formic acid and maintain the pH at or below 4.0.

The mixture must be stay for at least 24 hours before starting the next treatment phase.

Water

The disinfection of water is carried out by using disinfectants such as chlorine, chlorine dioxin or ozone and also by ultra-violet treatment or by physical processes such as membrane filtration.

Compared to chlorine, ozone provides good disinfection, without the risk of chlorinated by-products or taste defects (Anand *et al.,* 2014).

Meat products

Meat by-products, canned food or cured meats, are generally kept cooked. Two types of treatments are carried out for this type of products. Pasteurization carried out at temperature below 100°C, which allows the destruction of vegetative forms of bacteria such as *Salmonella*, and appertization, heat treatment carried out at a temperature above 100°C, necessary for the destruction of sporulating bacteria such as *Clostridium botulinum*.

CONCLUSION

Salmonellosis is one of the major food born diseases in the world, it constitute a public health emergency since the virulence and pathogenicity related, especially in the lower middle-income countries such as Morocco.

Poultry represents the first reservoir of *Salmonella*, from there, the study of the prevalence of *Salmonella* spp. at the level of broiler farming and at the other links (transport, slaughter, re-cleaning, cutting, staging) is necessary in order to know the levels of contamination that would be expected at carcass, cut, or finally on a ready dish to consume.

Additionally, hot countries have to carry out more studies in order to better understand the behavior and strategies to control of *Salmonella*.

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