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Food as Cause of Human Salmonellosis

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1. Introduction

Foodborne salmonellosis is still today a serious public health issue: very common in poor developing countries, due to the bad general hygiene conditions, it is also largely widespread in developed countries. In the latter, 95% of recorded clinical cases are foodborne (Liu *et al.*, 2011). According to EFSA epidemiological data (2011), in the European Union (EU) *Salmonella* is the second cause of foodborne disease after *Campylobacter* and it is still first in many EU States, such as Italy. Unlike *Campylobacter*, *Salmonella* often cause very large *multistate outbreaks* of food infection; this proves the greater resistance of this pathogen in the external environment and in food. In developed countries the main source of salmonellosis is still today food of animal origin, particularly meat (fresh and processed) and shell eggs. Also fresh fruits and vegetables can convey the bacteria to humans, as well as undrinkable water. *Salmonella* is quite resistant to adverse conditions and this allows them to persist in the environment and spread along the food chain, from the animals to the food of animal origin, or to plants that are fertilized with animal manure. Two species are currently registered into the genus *Salmonella*: *S. enterica* and *S. bongori*. The former is better adapted than the latter to live in the intestine of man and warm-blooded animals, whereas *S. bongori* travels in the external environment and is detectable in the intestinal contents of warm-blooded animals, so it is rare for it to be found in food for human consumption. The dangers for human health mainly arise from food contaminated with *Salmonella enterica*, which is often present in the intestines of livestock and pets, without causing any infection to the animals ("healthy carrier" condition). Humans can be healthy carriers of *S. enterica* in the intestine too. This may be a potential hazard to food hygiene, if the healthy carriers are the people involved in producing and handling the food. Usually a healthy carrier eliminates *Salmonella* in their faeces for several months after the episode of gastroenteritis through which they became carrier. In the case of *Salmonella* ser. Typhi, however, it has been demonstrated that humans can be asymptomatic carriers of the bacterium for decades (Weill, 2009). The genus *Salmonella* has more than 2,500 serotypes, and over 1,600 of these are within the *enterica* species, but not all serotypes have the same affinity for human and/or animals and they are not all found in the food that humans consume. Some serotypes (Typhy, Paratyphi A and C, some clones of Paratyphi B and Sendai) travel almost exclusively among men, and express their pathogenicity only when they infect a human being. Few serotypes travel exclusively among animals and do not infect humans, if not seldom (e.g. Abortusovis in sheep and Gallinarum-Pullorum in poultry). On the contrary, approximately 150 serotypes travel more or less constantly between the animal reservoir, the environment, food and man, starting from *Salmonella* ser. Typhimurium. Some serotypes, however, have a particular preference for some animal species: Enteritidis, Hadar, Heidelberg, Saintpaul,

Virchow, Senftenberg, Infantis and Kottbus find their main distribution channel in chickens, turkeys and ducks; Dublin and Bovismorbificans mainly infect cattle, while the Derby, Brandenburg and Panama serotypes frequently circulate among pigs (Weill, 2009). From the intestinal contents of livestock, the salmonellae can contaminate fresh meat, raw milk and egg shells. If the necessary hygienic precautions are not taken in the early stages of the production line (slaughter, milking, egg collecting), there is a risk that the salmonellae may then spread along each of their production chain, even polluting products such as cured meats, dairy products and egg-based dishes if they were made using raw milk or unpasteurized eggs. Moreover, through the faeces of animals and man, salmonellae can contaminate farmland, surface water flow and vegetables if they are fertilized with animal manure or dung that is not properly fermented. Vegetables, therefore, can be a source of disease to humans just like fresh meat, milk, shell eggs and by-products. Besides in animals, *Salmonella* can adhere well to the work surfaces, and from there spread to other foodstuffs by cross-contamination (Møretrø *et al.*, 2011). The examples are numerous and blatant: in the U.S. a major *Salmonella* ser. Enteritidis outbreak occurred and was associated with the consumption of industrial ice cream premix which was transported in tanks that had been used for carrying unpasteurized liquid eggs and were not properly sanitized (Hennessy *et al.*, 1996). An outbreak of salmonellosis due to *S*. Ealing caused by dehydrated powdered milk was traced back to the inadequate sanitization of production equipment (Rowe *et al.*, 1987). The thorough cleaning of work surfaces, both in food manufacturing facilities and in domestic kitchens, is therefore one of the main strategies for the prevention of foodborne salmonellosis (Møretrø et al., 2011). Generally, forms of gastroenteritis caused by non-typhoid *Salmonella* are moderately serious diseases with a quick recovery and without the need to resort to specific therapies. Although in some cases – when young children, elderly, or immunocompromised subjects are affected – salmonellosis may also lead to the patient's death (Pathan *et al.*, 2010). The severity of *Salmonella* infections can also be aggravated by the fact that in recent years more and more *Salmonella* strains have been spreading and they are resistant to one or more of the antibiotics which are widely used in human medicine, such as fluoroquinolones and third generation cephalosporins. In addition to the Typhimurium serotype, *Salmonella* strains which are multiresistant to many antibiotics have also been detected in the Agona, Anatum, Choleraesuis, Derby, Dublin, Heidelberg, Kentucky, Newport, Pullorum, Schwarzengrund, Senftenberg, and Uganda serotypes (Yan *et al.*, 2010). In most cases, human infection manifests itself through diarrhoea, persistent fever and abdominal cramps which appear 12 to 72 hours after the infection. The disease is self-limiting and clears up by itself within 4-7 days, but it has rather significant side effects: it takes months for the patient to regain proper bowel function and they can remain healthy carriers for months. In addition, chronic complications may occur such as widespread polyarthritis (Reiter's syndrome), ocular and urinary disorders, and even occasional cases of endocarditis and appendicitis. All these diseases are hard to treat even with antibiotics (Castillo *et al.*, 2011).

1.2 The infective dose "issue"
According to the regulations currently in force in the European Union, it is the manufacturer's responsibility to ensure the hygiene of their production processes on a daily basis, seeing to prevent any possible *hazard* that may contaminate food and be harmful to human health. The system used by food manufacturers to control processing hygiene in their facilities is the well-known HACCP system. In view of the fundamental principles of HACCP, if *Salmonella* contaminates a food, this is a *Hazard* because its presence could potentially cause harm to human health. It is, however, a hypothetical danger, as, for it to become real, the food has to

present some specific conditions. One of these is certainly the "minimal infective dose", i.e. the lowest charge that *Salmonella* must reach in the food for it to become dangerous to human health. Generally, it is accepted that *Salmonella* becomes truly dangerous for humans when it reaches in a food a charge of at least 10^4 cfu/g. However, it should be reminded that the bibliography reports some foodborne salmonellosis outbreaks caused by foods that contained less than 100 and sometimes less than 10 cfu of bacteria per gram of product. Fatty foods, such as cheeses, butter and chocolate, better protect the bacteria from the digestive enzymes in the stomach. In addition, the low water activity of these foods keeps the salmonellae in a latent phase, and this means that they do not proliferate in the food substrate, but can survive for very long time (Jansson *et al.*, 2011; Finstad *et al.*, 2011). The infective charge in one episode of salmonellosis which occurred in Canada and was caused by chocolate was estimated as low as 0,005 cfu/g (Komitopoulou & Penaloza, 2009). It is important to underline that the foods contaminated with Salmonella do not usually show any modification in their sensory characteristics even though the pathogens within have reached very high levels, concretely harmful to human health (Lindhardt *et al.*, 2009).

1.3 Epidemiology of foodborne human salmonellosis in the EU

According to the latest "European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks" (EFSA, 2011), in 2009 in the 27 EU Member States, the health authorities in charge have reported a total of 108,614 confirmed cases of human salmonellosis, with a prevalence of 23.7 cases/100,000 population. If we compare these levels with their equivalents reported from 2005 onwards, we discover that in the 2005/2009 period the cases of human salmonellosis have considerably dropped, estimated at -13%. In comparison, cases of campylobacteriosis have increased by +12%. In particular, between 2008 and 2009 there was a sharp decline in clinical cases of human salmonellosis caused by *Salmonella* ser. Enteritidis. All this indicates that the efforts made by health authorities and policies of individual EU states are obtaining positive and effective results. Furthermore, if we analyze the data regarding the spread of *Salmonella* among farm animals, we can find out that the importance of *Salmonella* as a cause of human foodborne disease is decreasing, also thanks to the decline in the spread of bacteria among livestock, starting with fowl. The decline in cases of foodborne salmonellosis among human beings does not tend to be consistent or regular in all 27 EU Members. The variations in the epidemiological pattern can be noticeable from one State to another. 10 states recorded a significant decline in cases; for 14 other states (including Italy) the epidemiological situation of human salmonellosis in food has remained essentially stable over the past five years, while Malta reported a sharp rise in cases (+24% compared to 2008), in contrast with the rest of the EU countries. Scandinavian and Central European countries are among the member states with the highest prevalence of human cases of foodborne salmonellosis while prevalence of salmonellosis among the population reported by the states bordering the Mediterranean are well below the previous. Epidemiologists interpret this as a sign of the single EU members' health authorities' increased awareness about the health of the populations under their responsibility. This increased attention to identify and report cases of foodborne salmonellosis explains the higher prevalence of human cases of salmonellosis in some northern European countries compared with the levels observed in Southern European countries. In most EU states food salmonellosis is a disease that patients contract "in their own country". Only Sweden, Finland, Denmark and the UK count a number of cases imported from abroad because they were contracted by people when they were out of the country. It should, however, be pointed out that some of the EU countries were not able

to ascertain and report to the EFSA the proportion of "national" cases of salmonellosis and those "acquired" from abroad. We would like to recall that in 2005 the EU issued the 2073/2005 (EC) Regulation which identified the *food safety criteria* for some of the major food groups most at risk of transmitting diseases to man. *Salmonella* was adopted as a parameter for the safety of fresh meat and products derived from it, raw milk and dairy products made with it, edible bivalve molluscs, as well as for pre-cut fruits and vegetables. In accordance with the EU provisions, *Salmonella* must be absent from 25 or 10 grams of examined sample of these foods in order for them to be destined for human consumption. In the EU which foodstuffs did not comply with this criterion and exceeded it? In 2009, as in 2008, the highest percentage of non-compliance was found in food derived from fresh meat, and particularly from minced meat and meat preparations containing chicken or turkey (8.7% of the total non-complying foods). Secondly, in order of prevalence, are bivalve molluscs and echinoderms, which are often traditionally consumed raw or hardly cooked (3.4% of all samples). Much less at risk are currently liquid eggs which go through a pasteurization process before entering the food manufacturing industry. Some concern arises from the fact that there are rather large percentages of non-compliance even among meat preparations for raw human consumption (the samples tested positive for *Salmonella* during official tests ranged from 1,2% to 1,7 % of the total tested samples).

2. Animals as *Salmonella* reservoir

The transmission cycle of *Salmonella* to humans through food presents many complexities because it involves animal reservoirs, vector food and the environment (Graziani *et al.*, 2005). Mammals, birds, rodents, reptiles, amphibians and insects act as environmental reservoirs of *Salmonella* and can transfer the pathogen to man (D'Aoust, 2007). On intensive farming facilities the role of the "healthy carriers" is important: even if they do not show any symptoms of the disease, they contaminate the environment and contribute to spreading salmonellae on the farm, sometimes creating endemic situations. The absence of symptoms in most of the infected animals and the technical difficulties in detecting the carriers during the inspection of the meat cause a continuous contamination of foods of animal origin.

Graziani *et al*. (2005) argue that various *Salmonella* serotypes may prefer various animal species: some are considered specific to one animal species (S. Gallinarum in chickens), others are defined as "host-adapted" because they prefer one host over another (S. Dublin for cattle, S. Enteritidis in egg-laying hens, S. Hadar in birds); on the other hand, other serotypes, such as S. Typhimurium, are ubiquitous. The role as reservoir is played by many animal species, but poultry and pigs are the predominant reservoirs for *Salmonella* (Cantoni & Bersani, 2010). In birds, species-specific serotypes are present, such as S. Pullorum and S. Gallinarum (Cantoni & Ripamonti, 1998), as well as host-adapted serotypes, such as S. Hadar and S. Enteritidis in chickens in Italy, while S. Blockley is found more predominantly in turkeys (Graziani *et al.*, 2005). The importance of broilers and other farm birds as *Salmonella* reservoirs should not be underestimated (D'Aoust, 2007). Although *S*. Pullorum and *S*. Gallinarum have been eradicated from industrial production thanks to *in loco* monitoring and eradication programs in reproducers, it is known that infections by S. Enteritidis and S. Typhimurium have been quite common in farm birds recently, therefore strict hygiene rules must be followed to prevent the contamination of finished products.

For pigs, the pathogenic salmonellae are *S*. Choleraesuis and S. Typhi suis (Cantoni & Ripamonti, 1998). Over the past ten years a marked increase in the prevalence of *S. enterica*

serovar 4, [5], 12:i- has been observed in many European countries (Hopkins *et al.*, 2010). It is resistant to ampicillin, streptomycin, sulphonamides and tetracycline in food-borne infections, in pigs and pork. The results indicate that genetically related strains of serovar 4, [5], 12:i:- of the DT193 and DT120 phage types with resistance to ampicillin, streptomycin, sulphonamides and tetracycline have emerged in many European countries and that pigs are the likely reservoir of the infection. A survey by the European Food Safety Authority has established the prevalence of *Salmonella* in pigs for slaughter in the EU-25 plus Norway (EFSA, 2008). This survey, as well as discovering that one pig every ten is affected, also identified the prevalent serotypes in infected pigs (*S*. Typhimurium and *S*. Derby), the same ones as in the cases of human infection.

Cattle are often colonized by S. Dublin and S. Typhimurium, with infections that vary in duration and clinical manifestation (Graziani *et al.*, 2005). Cattle are particularly susceptible to infection by *Salmonella* in the first weeks of life (Cantoni & Ripamonti, 1998). S. Dublin can stay in the host for a long time, in some cases all its life and often causes serious bouts of illness (Graziani *et al.*, 2005). As healthy carriers, they can pass S. Dublin and S. Typhimurium in their faeces, and those can remain viable in the outside for at least six months (Cantoni & Ripamonti, 1998).

In the meat-processing industry, eggs and poultry meat are the main groups of raw materials which usually carry *Salmonella* (D'Aoust & Maurer, 2007) and in many States they overshadow other sources such as pork, beef and mutton as a means of infection (WHO, 1988). To conclude, we can say that the biological cycle of *Salmonella* spp. is complex (see Table 1) and involves animals, environment and food (D'Aoust & Maurer, 2007), and that animals act as the most important reservoirs for its conservation (Graziani et al., 2005).
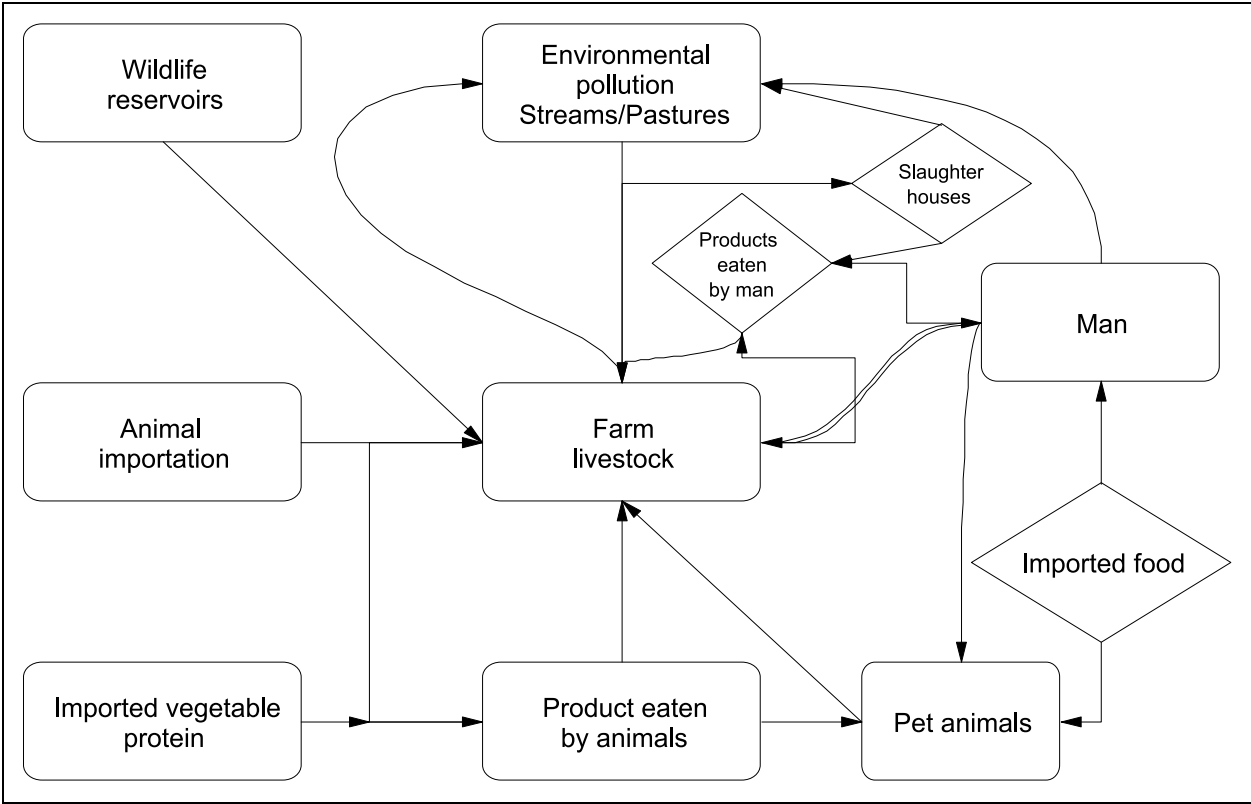

Table 1. *Salmonella* life cycle and transmission to humans (adapted from WHO, 1988).

3. Dynamics of the *Salmonella* population: Ecological factors

Foods are generally considered ecosystems made up of a habitat and a community of living organisms (biocenosis) that can influence each other and, in turn, be influenced by the habitat itself. Given the minute size of the microorganisms, the environment that affects them, at least in a solid matrix, is very small – of the order of a few millimeters or centimeters – so very heterogeneous physical and chemical conditions can exist in food (different conditions between the surface and the inside). In addition, a succession of microbial communities can be observed in food. The original microbial load, largely depending on the initial sources of contamination, is then replaced by new microbial communities that depend on the set of factors that appear during production and conservation processes. The factors that influence the development and survival of contaminating microorganisms are: (i) intrinsic factors, i.e. the characteristics of the food, arising from its composition or structure, pH levels, water activity (A_w) and redox potential (OR-value); (ii) environmental, extrinsic factors which come into action during the processing or storage of the food (storage or treatment temperature; relative humidity, light, storage environment); they may affect the intrinsic factors; (iii) implicit factors derive from the interaction between populations during manufacturing or storage. They can either be positive, such as mutualism and commensalism, or negative, such as competition, antagonism and parasitism.

Although it is possible to control the growth or survival of an individual or a group of microorganisms acting on only one of these factors, this is not always desirable, because it could have excessive consequences on the sensorial and nutritional qualities of food. As modern consumers are increasingly in demand of foods that look "natural" or "fresh", that are safe and have a relatively long shelf life, it is often necessary to act using a combination of factors, each of which is present at sublethal levels, but that, together, ensure the desired level of control. Therefore, instead of using a single barrier, combinations of barriers are used (the so called "hurdle effect") which cause, if the exposure to these conditions is prolonged, such damage that the microorganisms irreparably lose the ability to multiply, reaching their inactivation. *Vice versa*, if the microorganisms are subjected to lower levels of stress (sublethal conditions), they can adapt by activating a number of protection mechanisms, synthesizing proteins and other substances that improve their resistance to the stress in question or different stress. In recent decades, specific mathematical models have been implemented to describe the phenomena such as the growth, the production of metabolites and the death of the microorganisms found in various conditions, useful both for the conservation and for the hygienic safety of food. Predictive models, in particular, see to formulate mathematical models using adequate experimental designs which should provide information about the danger or conservation of food and about the possibility of growth, death or production of a toxin from pathogenic microorganisms. So, in view of the previous observations, we can predict microbial behaviour in similar environmental conditions (Ross & McMeekin, 1994). These approaches, however, are not disadvantage free, such as the variation of strains and the biomolecular knowledge for understanding which factors are responsible for pathogenicity. As regards *Salmonella* the ranges of the factors that favour their growth, death or survival are shown in **Table 2**.

Conditions	Minimum	Optimum	Maximum
T °C	7.0 (5.2 *)	35-43	46.2 (49.3 *)
pH	4.00 (3.80 *)	7.00-7.50	9.50
A_w	0.940 (0.900 *)	--	--
Tolerance to salt (%)	--	--	4

Table 2. Limits and optimum growth in relation to intrinsic and extrinsic factors for Salmonella spp. Notes. *: Some serotypes. (Source: ICMSF, 1996; amended).

Temperature

In particular, we can say that the minimum temperature for the growth of *Salmonella* is 7 °C (at 8 °C generation time is 22-35 hours); under 15 °C its development is still low. The storage of food at temperatures below 5 °C therefore prevents the multiplication of all serotypes; the only one able to develop up to 5.3 °C is *S*. Heidelberg (Matches & Liston, 1968). The highest mortality occurs during the slow freezing phase (0 to -10 °C), while in the deep-freezing one, for reaching temperatures below -17 °C rapidly, its survival is more likely, as damage to the cell membrane is minor. However, freezing does not guarantee the destruction of *Salmonella*: they have been found in frozen foods stored for years (ICMSF, 1996; Farkas, 1997), due to the changes and the production of cold shock and cold acclimation proteins (Scherer & Neuhaus, 2002). Maximum development temperature is 49.3 °C, beyond which *Salmonella* begin to die due to the denaturation of cell wall components and to the inactivation of heat-sensible enzymes. Although a temperature of 55 °C is sufficient to kill them, the legal limit for the storage of cooked foods meant to be eaten hot is normally 63 °C. *Salmonella* is not particularly resistant, so a pasteurization process is more than enough to destroy it. Several authors agree that the most heat-resistant serotype is *S*. Senftenberg 775W which registers D_{65}= 0.29-2.0 and D_{60}=1.0-9.0 when the substrate is in normal conditions, but the D value decreases if you move away from the optimal range for growth. Finally, its z value is 5,6-6,4 (°C). Resistance to heat is influenced by other factors, such as:
1. water activity: the lower it is, the greater the pathogen's heat resistance, since the presence of water favours the thermal break of the peptide bonds and in their absence more energy is needed for achieving the same result;
2. the composition of the food, and its fat content, which enhance its resistance, as well as the glycerol or sucrose contents;
3. pH levels, which, if maintained at around neutrality, allow for greater heat resistance of the pathogen, whereas sensitivity increases if it is lowered or raised;
4. the age of the microbial cells, since the young ones are more sensitive to heat in logarithmic growth phase;
5. adaptation to high incubation temperatures, for a genetic selection that favours the development of strains which are more resistant to heat (Jay, 1996).

Water activity (A_w)

Minimum water activity is 0,940, below which multiplication does not cease, but the bacterial charge decreases, without disappearing though. *Salmonella* can survive for long periods in conditions of dehydration. This was detected several times in sweets, including chocolate, which led to outbreaks of food infection (Werber *et al*., 2005). In fact, the high fat and sugar content of sweet, may lead to a protective effect against it. Of course, in chocolate,

there cannot be any growth, but rather a sublethal stress which leads to an adaptation of the pathogen (Jasson *et al.*, 2011). In the presence of NaCl concentrations between 3 and 4%, the development of *Salmonella* is usually inhibited. However, it appears that the inhibitory action of salt increases with increasing storage temperature. Variations depending on the serotype can be noticed (ICSFM, 1996).

pH value

The minimum pH is 3.80, but it all depends on the type of acid used, among which acetic acid seems to be more effective. Over the past twenty years, the survival of *Salmonella* under varying conditions of acid stress (Acid Tolerance Response ATR) has been extensively studied, especially regarding sublethal exposure with organic acids, which make the pathogen adapt to the acid used. The complex molecular mechanisms and environmental factors involved in ATR have been studied. An interesting discussion on this topic can be found in the article by Álvarez-Ordóñez *et al.* (2011). The increase in resistance to acids is very consistent, not only for the chances of survival of *Salmonella* in food, but also because it can lead the pathogen to resist to gastric pH (<1.5) and thus pass through the intestine unharmed. Generally speaking, we can say that *Salmonella* is very sensitive to acetic acid and lactic acid, while it is much more resistant to citric acid, used to acidify foods. In turn, these acids are more active if storage or treatment temperatures are close to the pathogen's minimum or maximum values of growth. Finally, we also have to underline that the acidification and/or heat treatment should not be applied to food in sublethal conditions, in order to avoid adaptation phenomena of pathogenic strains to the same treatment or even to different treatments (salt, water activity, etc.). Leyer & Johnson (1993) tested a strain of adapted to acid *S.* Typhimurium by constantly lowering the pH, finding out that the adaptation was not only due to the rebalance of intracellular pH, but also to a change in membrane proteins and not in the lipopolysaccharidic component.

Disinfectants

An incorrect method of disinfection and sanitization can make *Salmonella* persist on tools and utensils used in the food industry and kitchens, with the ability to form *biofilm* and, therefore, enable the spread of the pathogen.

Møretrø *et al.* (2009), using a treatment with a concentration of 100 ppm chlorine or 50 ppm of iodine for 15 minutes, noticed a biofilm can be completely removed, while with sodium hypochlorite (approximately 400 ppm) or cationic surfactants (benzalkonium chloride) for 5 minutes, *Salmonella* biofilm can resist on stainless steel surfaces. 70% ethanol for 5 min. is unable to remove the biofilm (Ramesh *et al.*, 2002).

4. *Salmonella* in vegetables

Compared to foods of animal origin, which are usually consumed once cooked, fruit and vegetables are mostly eaten raw and therefore a significant part of foodborne outbreaks due to the consumption of raw vegetables has been attributed to *Salmonella* (Cantoni & Bersani, 2010). Animal faeces and irrigation water are the main ways for *Salmonella* to spread to crops (Islam *et al.*, 2004). The water can contaminate the food if it is used for irrigation, for washing or for handling it (Rondanelli *et al.*, 2005). The salmonellae present in not perfectly ripened manure or in irrigation water invade the plants by gripping to the roots or contaminating the leaves (Cantoni & Bersani, 2010). Studies headed by Professor

Gadi Frankel (2008) of the Imperial College, London, UK, have revealed how salmonellae use their flagella to stick to salad and basil leaves. The results were presented at the 21st ICFMH International Symposium "Food Micro 2008" held in Aberdeen. This ability to attach itself to vegetables is described for a certain strain, *S. enterica* ser. Senftenberg, but not for *S.* Typhimurium (Frankel, 2009). Increased understanding of the mechanism that pathogens such as *Salmonella* use to adhere to vegetables is important if scientists are to develop new methods to prevent this type of contamination and the disease it causes (Berger *et al*., 2010). Schikora *et al*. (2008) have shown that *S.* Typhimurium, until now considered dangerous only for animals, can be a real danger for the vegetable kingdom too. Like any other plant pathogen, *S.* Typhimurium triggers the plant's immune defences and does not just cover the root surface, but enters physically into the plant's cells. The researchers attached a fluorescent probe to the bacterium and injected it, following its route: in just 17 hours the root cells were infected. Moreover, the infection later occurred simply by placing the plant (*Arabidopsis*) and the bacterium in the same liquid. *Salmonella* strains were detected in: aubergines, green salads, fennel, lettuce, onions, mustard, orange juice, pepper, parsley, spinach, strawberries, tomatoes, watermelons, coconuts, cereals, barley, chocolate and soy sauce (Cantoni & Bersani, 2010; Cantoni & Ripamonti, 1998). Today more and more ready-to-eat (RTE) vegetables are available in supermarket fridges because they are offered to the consumer as a convenience food, every part can be used, and, being already washed, peeled and chopped, they are quick and easy to prepare (Catellani *et al*., 2005). For their packing, various techniques are used, such as modified atmosphere packaging (MAP) – the gaseous composition of which varies according to the vegetable –, vacuum packaging, and ordinary atmosphere packaging. For the first two methods, the product should be packaged at refrigeration temperature, while with ordinary atmosphere it just needs to be kept cool. CO_2 has the function to slow the breathing and the appearance of rotting, to inhibit pectinolytic enzymes and the development of *Pseudomonas* and other Gram-negative bacteria (Galli & Franzetti, 1998). Manvell & Ackland (1986) show that RTE vegetables can host various saprophytic microbial forms: 80-90% are Gram negative spoiling bacteria (including *Pseudomonas* spp, *Enterobacter* spp, *Erwinia* spp) and the rest are yeasts and moulds. If Good Manufacture Practices are respected, pathogens (*Salmonella* spp., *L. monocytogenes*, *E. coli* O157, enterovirus) or protozoa (*Giardia, Entamoeba, Cryptosporidium*) are detected only occasionally (Catellani *et al*., 2005). The study of *Salmonella* has dramatically contributed to the knowledge of the epidemiology of these infections. Large-scale distribution, particularly of fruit and vegetables, sets the conditions for events that touch a very wide area, involve the exposure of a big number of individuals, and that are difficult to recognize for lack of sophisticated surveillance systems that should involve international collaboration networks.

5. *Salmonella* in eggs and egg products

Eggs laid by healthy animals are generally safe to eat because if they kept in sound hygiene conditions they can be considered almost sterile inside, especially as regards bacterial agents of food diseases (Bozzo, 2008; Galli & Neviani, 2005). Nevertheless, *Salmonella* spp. is the most important pathogen transmitted by eggs (ICMSF, 1998). The natural defence factors that may affect the egg's infection by microbiological contaminations are:
- *physical factors*: cuticle, shell, shell membranes, viscosity of the albumen and chalazae,

- *chemical factors*: pH of the albumen, lysozyme, avidin, flavoprotein, protease inhibitor protein molecules (ovostatin, ovomucoid, cystatin, etc.).

In addition to the factors mentioned above, we must add the environmental protection factors that are related to hygiene: egg-laying place, litter, surfaces, air, handlings, shell, duration and means of storage. The eggs can, however, be infected transovarianly with *Salmonella* by sick hens or healthy carriers (Cantoni & Ripamonti, 1998). There are many cases reported in the literature in which *Salmonella* was detected in eggs laid by hens with ovarian localization of this pathogen (in this case we speak about "endogenous contamination") (Bozzo, 2008). Through good hygiene practices in breeding facilities, it is possible to limit the number of microorganisms on the shell, as more than 90% of the contaminations of various origins occur after egg laying (Gandini, 1993). These *exogenous contaminations* of the egg can occur at different times: during transport or packaging or during the shelling (Bozzo, 2008). There is evidence (EFSA, 2009b) to indicate that cross-contamination between egg shells may occur during the manufacturing processes (sorting of the eggs, packing, etc.). The probability of this cross-contamination depends on the percentage of eggs contaminated with *Salmonella*, and the prevalence of eggs tested positive for *Salmonella* is also affected by the type of technology used and hygiene practices applied. However, the authors argue that we lack sufficient data to evaluate the occurrence of penetration through the shell and the proliferation of *Salmonella* due to cross- contamination during processing and, therefore, to assess the risks for consumers. The factors that influence the passage of microorganisms into the egg are: dampness, the shell's degree of contamination, the age-related decline in physical defences of the hen and the type of dirt that causes changes in surface tension (Galli & Neviani, 2005). Table eggs are pointed at as a major source of *Salmonella* and egg refrigeration has been recommended as one of many possible measures along the food chain to reduce the incidence of salmonellosis in the human population (EFSA, 2009b). The panel of experts on biological hazards states that refrigerating table eggs to temperatures at or below 7 °C limits the multiplication of pathogens such as *Salmonella*. If the cold chain is maintained, starting the refrigeration already on the farm is the measure with the highest positive effect in order to limit the proliferation of *Salmonella*. Table egg refrigeration is another safety measure together with other steps taken on the farm and during the processing as part of an integrated approach. Interruption of the cold chain is a factor that increases the risk of condensation and this may increase the penetration of bacteria into the egg (Ricci, 2005). The Salmonella infection cycle in poultry farms is summarized in **Table 3**. As stated in the EU Summary Report on trends and sources of zoonoses, zoonotic agents and resistance to antimicrobiotics (EFSA, 2007), the reported cases of human salmonellosis in the EU, respectively amounted to 154,099 and 31.1/100,000 inhabitants. The report also indicates that the prevalence of *Salmonella* in table eggs was 0.8%. According to the opinion of the European Scientific Committee on veterinary measures related to Public Health on *Salmonella* in food products in 2003, eggs and food produced with raw eggs (unpasteurized) are among the food categories most likely to cause cases of human salmonellosis (EFSA 2009). In Sweden, de Jong & Ekdhal (2006) compared the EFSA data on the prevalence of *Salmonella* on European egg-laying hen farms and the prevalence of human salmonellosis, revealing a high linear correlation between the two aspects. The same study analyzed the cases of salmonellosis in travellers returning to Sweden after

having been to several European countries with different *Salmonella* prevalence. The research seems to confirm the existence of a clear causal link between the presence of salmonellae in the egg production chain and the human disease. In Spain, Soler Crespo *et al*. (2005) focused on foodborne infections associated with the intake of eggs and egg products between 2002 and 2003. These outbreaks alone would account for 41% of all the episodes of food poisoning recorded throughout the duration of the study. The risk factors most often identified by the authors are the storage of the products at excessively high temperatures, the consumption of raw foods and a too long wait between the preparation and the consumption of the food. **Table 4** shows some events in epidemic proportions observed in recent decades in various parts of the world. These epidemics serve as a constant reminder of the fact that food technology cannot always protect against infectious diseases that may result in large-scale epidemics (multistate outbreaks) (Winn *et al*., 2009). In the United States, however, the introduction of a program for egg safety and quality (egg quality assurance programs [EQAPs]) plays an important role in reducing disease by *S.* Enteritidis transmitted from eggs (Mumma *et al*., 2004).
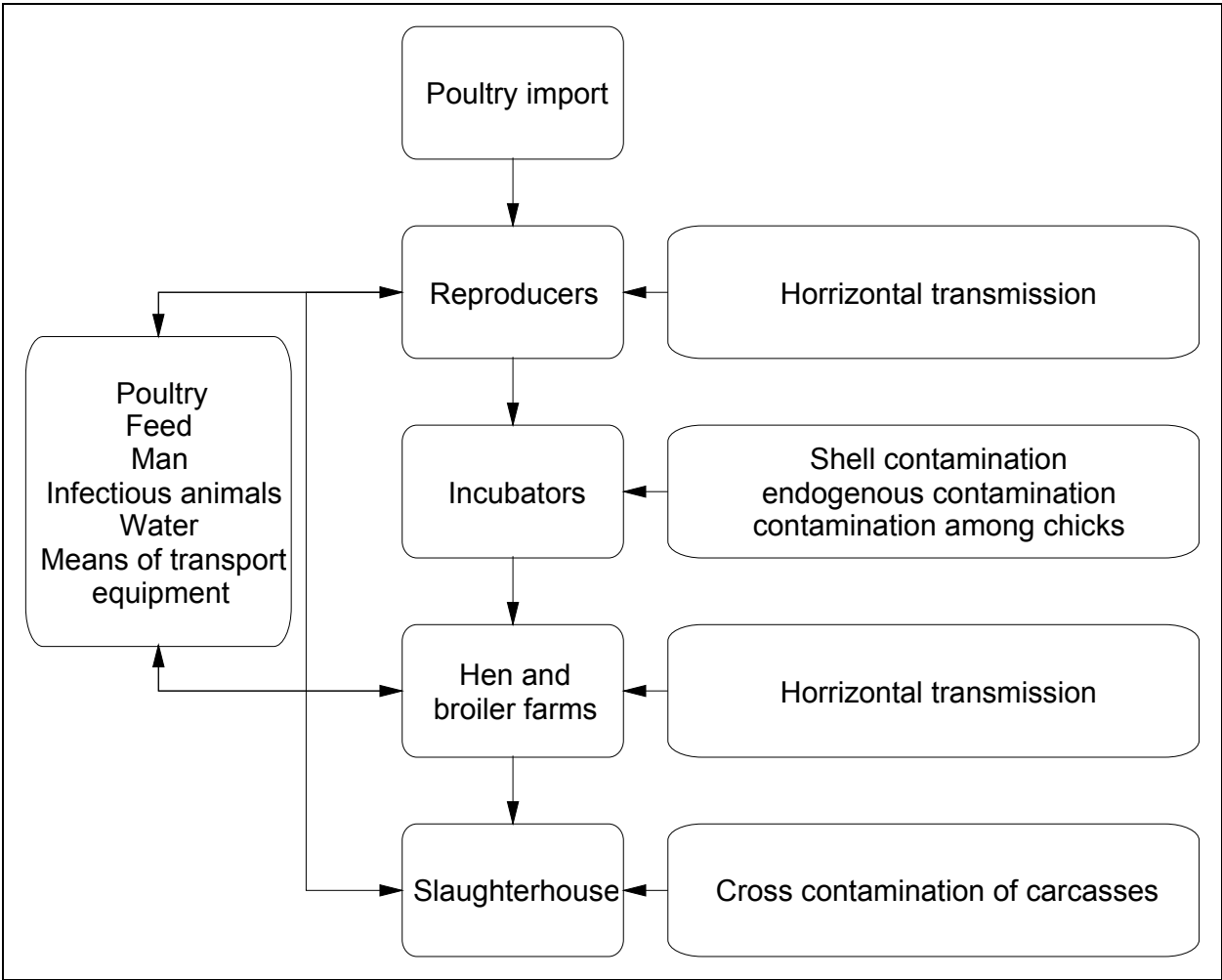

Table 3. *Salmonella* infection cycle in poultry farming.

Year	Country	Product	Serovar	Cases[a]	Deaths
1976	Spain	Egg salad	Typhimurium	702	6
1977	Sweden	Mustard dressing	Enteritidis PT 4	2865	0
1987	People's Republic of China	Egg drink	Typhimurium	1113	NS[b]
1988	Japan	Cooked eggs	*Salmonella* spp.	10476	NS
1993	France	Mayonnaise	Enteritidis	751	0
2001	United States	Tuna salad with eggs	Enteritidis	688	0
2001	Latvia	Cake/raw egg sauce	Enteritidis PT 4	19	0
2002	Spain	Custard-filled pastry	Enteritidis PT 6	1433	0
2002	England	Bakery products	Enteritidis PT 14	>150	1
2003	England, Wales, Scotland	Egg sandwiches	Bareilly	186	NS
2003	Australia	Raw egg mayonnaise	*Salmonella* spp.	>106	1
2003	United States	Egg salad kit	Typhimurium	18	0
2004	People's Republic of China	Cake/raw egg topping	Enteritidis	197	NS
2005	England	Imported shell eggs	Enteritidis PT 6	68	0

[a] Confirmed cases, unless stated otherwise.

[b] Not stated

(Adapted from: D'Aoust. J.Y. & Maurer J., 2007)

Table 4. Examples of outbreaks of human salmonellosis from eggs and egg products

6. *Salmonella* in meat and meat products

Meat includes all the edible parts of slaughtered warm-blooded animals, fit for human consumption. According to the EC Regulation 853/2004, this includes domestic ungulates (cattle, pigs, sheep and domestic equines), poultry, farmed lagomorphs (rabbits, hares), farmed game and hunted venison. It is called *fresh* meat if it has not undergone any treatment to extend its shelf life, except for the use of cold (refrigeration, freezing, deep freezing). Vacuum-packed meat and meat packed in a protective atmosphere are also considered fresh. Due to its chemical composition and to its intrinsic characteristics (A_w above 0.99, pH between 5.5 and 5.8), fresh meat is a good substrate for microbial growth. For this reason, cooling after slaughter is a critical point because it determines the microbiological quality of the product and must occur as fast as possible (internal temperature ≤ 7 °C, within 24-30 hours following slaughter). The flesh of healthy and unstrained animals is devoid of microorganisms in depth; but due to stress before slaughter, disease, or weakness, microbial contamination can occur and is defined as endogenous: pathogens, in particular starting from the intestine, spread into the blood due to the failing

immune system, and reach the muscles, lymph nodes and internal organs. Among these microorganisms, there may also be *Salmonella*, if it is present in the intestinal contents. In this case, after the analytical laboratory investigations requested by the Veterinary Inspector, carcasses must be confiscated and destroyed, as they represent a potential danger to the consumer. On the other hand, the main microbial contamination occurs during the various stages of butchering and cutting, as well as in the following stages, such as the preparation one (minced meat, sausages, kebabs, etc.) and processing (salami mixture), until the purchase and the preservation of meat products before consumption. This contamination, defined as exogenous, is inevitable, but, by applying good manufacturing practices, it can be successfully controlled. The slaughtering stage which can lead to greater contamination by *Salmonella* is the gutting, where the release of feces even if it is limited (from 10^{10} to 10^{12} cfu/g microorganisms depending on the animals) results in the contamination of more or less large parts of the carcass. The main animal species that can host *Salmonella* spp. in their intestine, in descending order, are farmed birds, pigs and cattle. Meat is no doubt the food that undergoes the greatest number of tests, imposed by strict rules: on-farm veterinary visits, certificates accompanying the animals during its transport to slaughter, *ante mortem* and *post mortem* inspections, the scalding of the carcass (domestic ungulates and big game) that makes it fit for human consumption; followed by tests in the next stages (butchering facilities, butcher shops, supermarkets, meat-processing facilities, etc.) on meat and internal organs (heart, liver, stomach, etc.). Nevertheless, to restrict the *Salmonella* issue in meat, it is important to act upstream of the chain of production, during primary production. Ever since the 1990s, for poultry, the WHO (1994a) indicated guidelines to follow in order to identify the infected farms, to keep the vectors that carry the infection under control (WHO, 1994b) and to apply prevention methods (WHO, 1994c). In more recent years, the EU has released surveillance systems with specific control programs to significantly reduce the problem of *Salmonella* on farms rearing breeding poultry, egg-laying hens, broilers, turkeys and pigs, both for breeding and for meat (EC Regulation 2160/2003, Appendix 1). In these farming facilities, it is necessary to keep under control the hygienic characteristics of raw materials and animal feed, environmental hygiene, rodents, overcrowding, animal welfare, etc. The contamination of food for animal feed can occur in the factory as well as on the farm by cross contamination (not properly sanitized utensils) or by means of vectors (rodents, insects). Against the spread of *Salmonella* Enteritidis on poultry farms, it is effective to use antimicrobial agents in the feed, such as organic acids; as well as adding to the drinking water mixtures of probiotic bacteria in the early weeks of life, during which the intestinal colonization by potentially pathogenic microorganisms is most likely. Another difficulty resides in the elimination of the pathogen from the environment through cleaning and disinfection carried out after sending the animal to slaughter and before the arrival of the next cycle. Therefore, a good approach for controlling infection on the farm is definitely that of adopting prophylactic measures with serological monitoring and an accurate microbiologic testing of environment and faeces, trying to avoid the overuse of antibiotics in animals, which, on the other hand, can decrease their own organic resistance against *Salmonella*. In pigs, *Salmonella* can also be found very frequently in the tonsils, contaminated orally together with the intake of food. According to Griffith *et al.* (2006), it is possible to detect it in the oropharyngeal secretions and transmission between animals can happen nasally, especially in case of overcrowding on the farm or in transport. In cattle, the increased susceptibility to infection may arise from errors in the formulation of the food that changes the rumen flora, thus favouring the development of *Salmonella*. The EFSA report on

the progress of zoonoses and foodborne diseases (EFSA, 2011) shows that the verification of *Salmonella* in intensive European poultry farming facilities went down in 2009 compared to previous years. Greece (7%), the Czech Republic (11%) and Hungary (32%) were the states where the detections were greater, respectively for roosters, egg-laying hens and broilers. In the first 2 types of farming facilities, the most detected serotypes were: *S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Virchow and *S.* Hadar. On pig farms, the data collected among the member states are not homogeneous about sampling time in the production chain (faeces on the farm, lymph nodes in the slaughterhouse): the only countries that submitted full data were Estonia (0.9% on the farm and 8% positive in the slaughterhouse) and Norway (0% in both cases). However, the EU estimated that the presence of *Salmonella* in intensively farmed pigs in 2009 varied from 0 to 64% (on average between 26 and 31%). S. Derby was the most detected serotype. For all other animals, although gathered data are few, the presence of *Salmonella* is low both on the farm and in the slaughterhouse. Finally, *Salmonella* was found in animal feed with a low incidence, ranging on average from 0.4% (for cattle) to 1% (for poultry), and the most contaminated products were meat and bone meal (1.4%). In later farming stages it is necessary to ensure that the animals arrive at the slaughterhouse in the best conditions possible (avoiding overcrowding, dirt on skin and feathers); it is also important to check, in addition to the above, the fasting time before slaughter, the temperature and the renewal of scalding water (poultry, pork) in order to limit the load of organic material and reduce the adhesion of *Salmonella* on the skin; it is also essential to check that the cleaning procedures, the maintenance of facilities and equipment are performed in optimal conditions. These checks should be supported by microbiological tests, in accordance with EC Regulation 2073/2005, to make sure that the slaughter took place in full respect of hygiene conditions and, if not, to review the process. Moreover, EFSA (2006) recommends that swine slaughter monitoring should provide for the research of the pathogen in the ileocaecal lymph nodes or in the meat juice. Decontamination of carcasses after slaughter can be useful for controlling pathogens, including *Salmonella*, but it can absolutely not replace poor hygiene during the slaughter. Some methods put forward, but not always accepted by the regulations, are the use of organic acids, such as acetic acid, which cannot be used for obvious reasons, and lactic acid, which, if used at concentrations of 1% v/v, affects S. Typhimurium well without affecting the colour or the flavour of the meat too much; also ozone mixed with the water used for showering poultry provides excellent results regarding the reduction of microbial load. In the USA, trisodium phosphate (TSP) can be used, an alkaline composite (pH 11) able to diminish the pathogen by 2 logarithmic cycles and bring down the contamination of poultry carcasses to under 5% (Gudmundsdottir *et al.*, 1993), but its disposal after use is a problem.

A very powerful physical method to reduce microbial load is γ-ray irradiation, which is not licensed in Europe. Doses of 3-5 kGy are employed, effective for the decontamination of fresh meat, the Enterobacteriaceae loads ensuing in a fall of 6 logarithmic degrees (WHO, 1994), without causing significant deterioration of the sensory quality of the treated products. As regards the meat of hunted wild game, the microbiological quality depends on where skinning and handling is carried out: on average, it was stated that the total viable count as well as that of fecal coliform are about 2 logarithmic higher in game eviscerated outdoor than the similar value of game eviscerated in slaughterhouse (10^5-10^8 vs. 10^3-10^6 cfu/g). Anyway, *Salmonella* is rarely found, especially if the wild game does not come into contact with domestic animals. Frogs imported from various countries are frequently contaminated with *Salmonella* (20-30%) belonging to most diverse serotypes and often exotic ones, according to the importing country.

Snails, however, although they are very often contaminated, rarely cause food-poisoning episodes as the way they are cooked ensures they are sanitized; nevertheless, they represent an excellent means for the germ to spread (Tiecco, 2000). Possible contaminations can also occur when the meat is subjected to various types of processing, such as "minced meat", defined as meat meant to be minced and to be sold as such, with the addition of salt up to a maximum of 1%, and "meat preparations", i.e. meat products to which seasonings or permitted food additives have been added or any treatment that does not alter the cellular structure of the core of the meat and does not change its characteristics as fresh meat. In both cases one can easily verify microbial contamination due either to the physical characteristics of the products, or to whether other potentially *Salmonella* carrying ingredients (vegetables, eggs, spices, etc.) are added. It is therefore important to keep the temperature constantly below 4°C, staff hygiene rules must be respected, as some staff members may be asymptomatic carriers of *Salmonella* and, most importantly, cross contamination must be prevented throughout the production chain and after cooking the meat, because this is the major problem for *Salmonella*. The processes of salting, curing, cold smoking, antimicrobial preserving additives and lactic fermentation the meat undergoes for becoming cold cuts are not able to completely eliminate *Salmonella*, but do cause the numbers to fall, sometimes substantially, which protects the consumer from food poisoning (<100 CFU/g). Heat (pasteurization, cooking, hot smoking), on the other hand, if it is done properly, is a good clearing method. But, without respecting basic technological principles, the previous operations will be completely useless. Many researchers agree that *Salmonella* usually contaminates food with a very low charge, mostly <10 cfu/g, but in the case of a human in normal immunological conditions, in order to be effective, an infection requires the ingestion of a fairly large charge, more than 10^4 cfu/g of food. As a result, it is necessary for the bacteria to find adequate conditions in the substrate to duplicate more or less rapidly. The "hygiene package" regulations introduced the obligation for the owners of manufacturing and processing factories to arrange and implement self-control procedures, to perform microbiological tests on the finished product and to indicate on the label if such meat, other than poultry, is to be subjected to adequate cooking before consumption.

Researches in EU in 2009 (EFSA, 2011) showed that *Salmonella* was found most frequently in raw turkey meat (8.7%), chicken (5.4%), pork (0.7%) and cattle (0.2%). In general, at the pig slaughterhouse, *Salmonella* was detected between 0 and 14%, with a higher incidence in Belgium where a very meticulous sampling method is carried out. According to the EFSA, many episodes of human food-borne illnesses are attributable to pork. Minced meat and meat preparations, especially those intended to be eaten raw, are most often contaminated by bacteria (5.5% positive) and therefore do not meet European sanitary standards. In retail shops *Salmonella* was found in 3.5% of the analyzed samples, a decrease compared with the previous year. Finally, the analysis of the results shows that there is a prevalence of serovars Typhimurium and Enteritidis.

In conclusion, the major risk factors of salmonellosis due to meat are to be found in insufficient cooking, the consumption of raw meat (pork sausages) which was not processed properly (minced horse meat, carpaccio, tartare, etc.), storage at inadequate temperatures, and cross contamination, if the product ready for consumption is in contact with other raw foods or dirty utensils. On the other hand, the staff's role, whether infected or carrier, appears to be less important, as it has been proven that, if Good Manufacturing Practice (GMP) is applied to food, such as careful hand washing, the risk of conveying infection is kept well under control.

7. *Salmonella* in raw milk and milk products

Nowadays, according to the EC regulations in force, we can obtain milk for human consumption from all mammalian species, without exception, provided that the animals are reared and milked when they are in good health and nutrition conditions (EC Regulation 853/04). In developed countries the milk that humans use as food is almost always cow's milk; much more seldom we also consume sheep's, goat's and buffalo milk. In poor developing countries, in addition to cow's milk, buffalo, sheep's and goat's milk is also regularly drunk. In different geographical areas and with varying eating habits, along with "traditional" dairy species, man also employs various other animal species (camels, dromedaries, horses, reindeer, etc.) as a source of milk. Thanks to its chemical composition, milk is an almost ideal food for humans and for this reason it is part of the daily ration of most of the world's population. Over time the different peoples on Earth have developed a remarkable range of food products that use milk as raw material: in the world today there are approximately 1,600 different types of cheeses and over 100 different types of fermented dairy products. From raw milk many kind of milk products are obtained, such as pasteurized or UHT milk, cheese, fermented milks and probiotics, ice cream, butter, ricotta and milk drinks or whey. This wide range of food is obtained by subjecting raw milk to one or more technical processes that change the components of milk and its rheological properties to a greater or lesser extent. These "treatments" may be the addition of salt and the removal of water (*seasoning*) or the addition of natural enzymes and/or milk ferments that trigger these complex biochemical processes that we call *ageing* of cheese or fermented milks. *Salmonella*, as well as other pathogenic agents of foodborne disease, can contaminate raw milk: (1) directly inside the mammary gland (very rare event); (2) during milking, because the bacteria are often present in the faeces of milk animals and on their coat; (3) after milking, because salmonellae can contaminate work surfaces with which the raw milk comes into contact; (4) in subsequent phases, still due to the presence of *Salmonella* on work surfaces and/or cross contamination. The fate of salmonellae in milk and milk products widely depends on the antimicrobial effects the different transformation processes may have on the bacteria, as bactericidal effect or more simply bacteriostatic effect. This explains why in developed countries cases of human salmonellosis caused by the consumption of dairy products and milk are much rarer than those caused by the consumption of fresh meat or fish products (Jayarao & Henning, 2001). Raw milk, of course, represents an exception: in recent years it has caused a number of fairly many outbreaks of human salmonellosis (Newkirk *et al.*, 2011). We must not forget that in recent years in many European states the consumption of raw milk purchased directly from the dairy by means of automatic vending machines has greatly increased. The following study will, therefore, give information about the possible presence of *Salmonella* in raw milk and different products that are derived from it, focusing above all on the possibility for *Salmonella* to multiply in different dairy products. According to the epidemiology data of foodborne illnesses provided each year by the EFSA, milk and milk products are not by far the greatest sources of danger for consumers. Like in previous years, in 2009 too there were few cases of *Salmonella* detected in cow's milk. Only three EU Member States conducted specific tests on raw milk sold in vending machines: Austria (71 samples tested), Germany (173 samples) and Hungary (50 samples). *Salmonella* was never detected in any of these samples. On the other hand, as regards pasteurized or UHT milk, seven states reported data: Austria (30 samples), Bulgaria (30 samples), the

Czech Republic (135 samples), Germany (980 samples), Greece (26 samples), Hungary (85 samples) and Romania (57 samples). Again, none of the samples tested contained *Salmonella*. Italy reported that out of a total of 928 samples of cow's milk, 3 were positive for *Salmonella* and that 5 samples out of a total of 5,799 samples of milk from "other unspecified species" also tested positive for the pathogen. 11 member countries supplied EFSA with results of their investigations on cheeses, for a total of 23,023 samples analyzed. In the great majority of cases, the cheese samples proved to be negative for *Salmonella*, with the exception of Spain (4 positive samples out of a total of 424 samples tested), Portugal (2 positive samples out of 181 analyzed) and Italy (2 positive out of a total of 1,879 samples tested). As far as we know from the EFSA report, all the cheeses tested positive were semi-hard cheeses, and only semi-mature, and made from raw or thermised milk (i.e. heated to a temperature between 45 °C and 54 °C, no more). As far as butter is concerned, 7 member states communicated the results of their inspections; no case revealed the presence of *Salmonella*. Besides cheese, the only other product derived from milk which pointed out the presence of *Salmonella* was ice cream. Spain, Hungary and Germany reported the presence of the bacterium respectively in 13 samples out of 305 samples analyzed, 1 out of 140 and 1 in 2,626 samples, always taken in the production facilities. The presence of salmonellae in raw milk is widely documented in the literature, both in the collection tanks on the farms, and in the storage tanks in the food factories (Donaghy *et al.*, 2004; Tondo *et al.*, 2000). *Salmonella* may be present in raw milk ever since milking because the bacterium is present in the mammary gland, but this occurs very rarely. Mastitis due to *Salmonella* is a very rare condition in dairy cows, but it is reported. We know that different *Salmonella* serotypes can colonize the mammary gland and lead to the excretion, at the same time as the milk, of bacterial loads that can extend up to 3.3 log10 cfu/ml (Fontaine *et al.*, 1980). Furthermore, *Salmonella* can pass from animal to animal at the time of milking, both through the milker's hands, and through polluted parts of the milking machines (Bergonier *et al.*, 2003; Vautor *et al.*, 2003; Zadoks *et al.*, 2002; Zschöck *et al.*, 2000). Much more often, however, salmonellae contaminate raw milk in the stages that follow the milking process, because the bacteria may be present on the various surfaces that come into contact with the milk being collected. In particular, *Salmonella* (such as *Listeria monocytogenes* and verotoxigenic strains of *E. coli*) can enter the milk through the traces of animal faeces in the environment (Van Kessel *et al.*, 2004). This factor of pollution, in turn, is influenced by the prevalence among dairy cows of *Salmonella* healthy carriers, which can evacuate various loads of the pathogen more or less frequently. In this regard, it is estimated that the U.S. dairy cows can be healthy carriers of *Salmonella* in their faeces with a prevalence that ranges from a minimum of 2% to a maximum of 27.5% of the animals tested (Kabagambe *et al.*, 2000; Losinger *et al.*, 1995, Wells *et al.*, 2001). What can be the prevalence of a batch of raw milk tested positive for *Salmonella* ever since the milking phase? In view of the data that we can gather from the literature, we can estimate that the batches of raw milk straight after milking can be positive for *Salmonella* from a minimum of 2.6% to a maximum of 25.3% (Jayarao & Henning, 2001; Murinda *et al.*, 2002; Zhao *et al.* 2002). Compared to other pathogenic microorganisms such as *L. monocytogenes*, salmonellae are not very resistant in the outside, so it is not very frequent for the work surfaces in the production plants to transfer salmonellae to the product. Nevertheless, in theory, *Salmonella* can survive for long on any work surface and then pollute the cheese curd which is meant to become cheese. This justifies the episodes of foodborne infection caused by processed dairy products, such as

milk powder and cheeses made with pasteurized milk. **Fermented milks** can be divided into two kinds: (i) acid, if their production is based on homolactic fermentation, (ii) acid-alcoholic, if the starter strains used for fermentation are of the heterofermentative type. In case (i) the product will only be acid, while in case (ii) besides the presence of acid there is a fair amount of ethyl alcohol which enhances the food's antimicrobial effect against *Salmonella*. Their production process usually starts from pasteurized milk. Furthermore, milk is caused to coagulate by using acid, by adding selected milk ferments that produce large amounts of lactic acid or other organic acids and possibly ethyl alcohol, with a drastic drop in the substrate's pH which makes the casein coagulate. The presence of high loads of lactic acid bacteria, coupled with low pH levels (4.0 to 4.1 on average) and A_w mean that yogurt and other fermented milk products are a very unfit food matrix for allowing the growth and even the survival of *Salmonella*.

Cheese is among the foods which are less likely to cause salmonellosis in humans due to their production process (Little *et al.*, 2008). Nevertheless, in 2008 it was responsible for 0.4% of all episodes of illness reported in the EU (EFSA, 2010). In addition, several cases of salmonellosis caused by the consumption of cheese contaminated with *Salmonella enterica* are reported in the bibliography. The problem is that despite the fact that the production process poses several obstacles to the survival and multiplication of salmonellae, we eat cheese without further heat processing. Moreover, cheese often does not carry pathogenic microorganisms in its inside, but rather on its surface. This may result in the transfer of *Salmonella* and other pathogens to domestic working environments, thus favouring cross contamination, which in turn enables the outbreak of foodborne illnesses (Kousta *et al.*, 2010). The bibliography gives at least a dozen episodes of salmonellosis caused by the consumption of cheeses made not only with raw milk, but also with pasteurized milk. This means that in many cases the milk used to produce cheese is contaminated with *Salmonella* "after" its pasteurization, since this is largely able to inactivate very high loads of the bacteria. Nowadays, HTST pasteurization is often used in the dairy industry (at least 72 °C for at least 15 seconds) and it can produce a drop of about 6 LOG-degrees in the original load of *Salmonella*, as demonstrated by accurate experimental investigations (D'Aoust *et al.*, 1988; D'Aoust *et al.*, 1987; Farber *et al.*, 1988). In particular, these studies showed that *Salmonella* can still be detected in milk heated up to 67.5 °C for 15 seconds, but not at higher temperatures. We need not forget, though, that *Salmonella*, just like *Listeria monocytogenes*, can penetrate into the milk somatic cells that can provide it with a slight protection against the effects of heat. It is not, therefore, possible to exclude *a priori* that in normally pasteurized milk it may still be possible to detect some salmonellae which survived the treatment itself, if it was not carried out at temperatures above 72 °C. In the past decades, salmonellae have caused a series of outbreaks of illness caused by the consumption of various types of cheese. As mentioned before, we can find several references in the literature to outbreaks of salmonellosis caused by foods that contain very low numbers of *Salmonella*. According to D'Aoust (1985) and Ratnam & March (1986), the literature documents cases of salmonellosis caused by Cheddar cheese in which the estimated infectious load proved to be under 10 cfu of *Salmonella*/g of food.

From the data we possess, we can therefore sum up that *Salmonella* may still be present in cheeses for human consumption, but with a prevalence which varies widely depending on several factors:

1. the type of raw material: cheese made with raw milk may contain salmonellae still alive and vital, while it is hard for those made with pasteurized milk to still shelter the pathogen, unless the contamination occurred after the pasteurization process,

2. the duration and type of ageing: in cheeses which mature for a short time, *Salmonella* is more likely to survive, because the maturing biochemical processes that have a good antimicrobial effect against pathogen are not yet established in the substrate. In cheeses that mature for over 60 days, on the contrary, the characteristics of the substrate that are obtained as a result of aging make the product unfit for the reproduction and survival of salmonella,

3. the microbiological quality of milk used to make cheese. Cheeses made with raw milk are not necessarily infected with *Salmonella*, if good hygiene conditions are maintained during the milking process and the ensuing manufacturing process.

As with many other types of foodstuffs, salmonellae can contaminate cheese coming from:

1. raw materials used in production, most likely from raw milk and less likely from other ingredients such as lactic acid starter and salt,

2. salt solutions (brine) used for salting certain products,

3. work surfaces in the cheese factories, including the air that circulates in various environments,

4. packaging materials in which is wrapped the finished product ready for sale (Temelli *et al*., 2006).

As regards in particular brines used to salt the cheese, Ingham *et al*. (2000) conducted experimental inoculation tests with *Salmonella* ser. Typhimurium to test the viability of the pathogen in the cheeses' brines. The researchers experimentally inoculated two cultures with *S*. Typhimurium and *E. coli* O157, mixed together, in three different brines containing 23% salt, with the addition of 2% of flour. The brines were then stored at 8 °C and 15 °C for 28 days. The same cultures were also inoculated into brines offered for sale, and then stored at 4 °C and 13 °C for 35 days. The load of the two pathogens immediately underwent a gradual decline during storage, but it is significant that the reduction was less noticeable in the brines stored at 4 °C compared to the ones stored at 13 ° or 15 °C. This study shows that *Salmonella* may still survive in saline solutions used for salting cheese, although with very small loads.

Compared to other pathogens such as *L. monocytogenes* and *Staphylococcus aureus*, *Salmonella* is much less often blamed as a source of illness due to the consumption of cheese. As a result, we do not have precise data as to the actual prevalence of *Salmonella* in cheese. We can, however, find some data on the persistence of salmonellae in cheese sold in retail food stores. The pathogen was detected in Turkey in various kind of cheese produced mainly in an artisanal manner with raw cow's, ewe's and/or goat's milk (Colak *et al*., 2000; Hayaloglu & Kirbag, 2007; Tekinşen & Özdemir, 2006), always in very low prevalence of the samples analyzed. On the other hand, we also have data documenting how salmonellae, potentially present in raw milk and/or in environments where milk and cheese are produced, are not so detectable in the dairy products offered for sale. For example, in Spain Cabedo *et al*. (2008) conducted a large study to test the microbiological quality of the cheeses of their land: they never detected *Salmonella* in any of the samples they analysed. In Britain, two studies conducted by Little *et al*. (2008) first in 2004 and then in 2005, showed that a total of 4,437 samples of various types of cheeses (fresh, semi-mature and mature, made with raw or pasteurized milk) never showed the presence of *Salmonella*.

Butter is produced by the mechanical churning of the cream obtained after centrifugation of cheese whey. It can be *sweet* if the cream is used as it is, or *ripened* if it comes from cream that was first matured with the addition of starter enzymes. In most cases, the raw material for butter is subjected to pasteurization in butter before being processed, but in some cases butter is obtained directly from the cream of raw, unpasteurized milk. It is clear that in this second case *Salmonella* may be present in the butter from the start of the making process because the raw material itself was contaminated. In the case of butter made from pasteurized cream, however, a possible contamination with *Salmonella* cannot be excluded, because the pathogen could infect the finished product through a secondary contamination. In the past decades, in fact, several episodes of human salmonellosis caused by butter contaminated with *Salmonella* occurred, but over the years these episodes have registered a sharp decline, due to the fact that producers dedicate more attention to production hygiene and to the fact that butter is now rarely made with unpasteurized cream. The EU has established with EC Regulation 2073/05 that "cheese, butter and cream made from raw milk or milk subjected to heat treatment at sub-pasteurization temperatures" should not contain even one living cell of *Salmonella* in 125 g (25 g in 5 units of the sample) of product throughout its shelf life. **Dried milk products** as a rule, these foods are products obtained after pasteurized milk is nebulized in towers where a very dry and hot air current circulates, but on the market you can find lyophilised products, i.e. put through the cold-removal of water, not involving the use of high temperatures. The sanitary characteristics of milk powders, therefore, is determined by: (i) the microbiological quality of the raw material, (ii) the conditions of the production process (with or without heat treatment), (iii) the possibility of the dehydrated/lyophilised product to be contaminated with salmonellae after its processing. Salmonellae are sensitive to normal temperatures applied in the production process of dried milk products, so it is logical to expect that such products are rarely at risk of containing *Salmonella*, unless they are contaminated after this process, during packaging or storage. In these cases, dried milk products may be a risk to human health, since salmonellae can survive for months in substrates with low water content, such as bone meal and powdered foods. The possible dangers of these products is also enhanced by the fact that such foods are usually meant for very young children, much more sensitive than adults to even minor loads of *Salmonella*. For this reason, the EU has established by law (EC Regulation 2073/05) that "powdered milk and powdered whey" should not contain even one living cell of *Salmonella* in 125 g of product throughout its shelf life. **Ice cream** is a complex food made of various ingredients, including eggs and milk, where water crystallizes, forming a homogeneous creamy mass, thanks to the high amount of fat. As such, also ice cream can be contaminated with *Salmonella*, if it is contained in the raw milk or appears in the manufacturing process. Over the past decades, in fact, many outbreaks of salmonellosis caused by the consumption of ice cream have been documented, but it was not always possible to establish with certainty whether the pollution came from the raw milk or from the eggs, which are also used raw. For several years now, the use of pasteurized milk and eggs has become a habit for producing ice cream, so the risk of *Salmonella* contamination in these products has been greatly reduced. But we must remember that ice cream, due to its almost always neutral or slightly acidic pH levels and to its high amount of free water (A_w), can be an excellent substrate for the survival and growth of *Salmonella*, if the latter managed to infect it. The risks to public health may be greater for

those who produce ice cream from raw milk. In recent years, in fact, this habit seems to have come back into fashion, under the pressure from consumers who take great pleasure in consuming food products from raw materials treated as little as possible. Regarding ice cream too, the EU has set specific criteria for *Salmonella*, which must be "absent" in 125 g of product. This law does not apply to ice creams "whose manufacturing process or composition properties eliminate the risk of *Salmonella*" as required by Regulation 2073/05.

8. Conclusion

All this makes it difficult to control and prevent these toxi-infections; as a result, it is necessary for epidemiologists, clinicians and microbiologists as well as veterinarians to collaborate in order to launch an integrated approach to solve the problem. In order to prevent the occurrence of salmonellosis, it is therefore essential to know which animals and/or which foods most frequently carry the pathogens which have led to sporadic cases or episodes of disease in humans. Epidemiological data should then be given special attention and consideration by meat producers and in general by anyone whose role it is to carry out investigations on food, as they can provide useful information regarding changes or additions to be made to the eradication plans against *Salmonella*.

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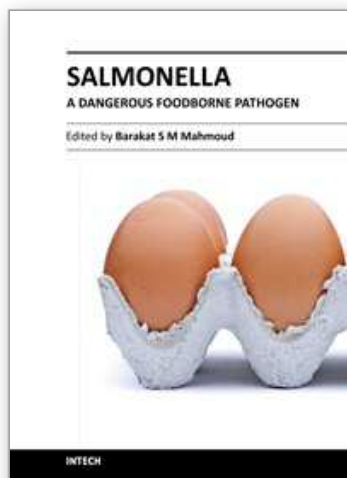
Salmonella - A Dangerous Foodborne Pathogen
Edited by Dr. Dr. Barakat S M Mahmoud

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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