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Prevalence, Risks and Antibiotic Resistance of *Salmonella* in Poultry Production Chain

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Abstract

Salmonella spp. are bacteria that cause salmonellosis, a common form of foodborne illness with major impact on human health and huge financial losses in poultry industry. The incidence of notified cases of salmonellosis has declined from a peak of 24 per 100,000 in 2009 to 20.4 reported cases per 100,000 population in 2013, with *S. enteritidis* and *S. typhimurium* being the most commonly reported serovar in EU. *Salmonella* spp. has been detected in a range of foods, and outbreaks have predominantly been associated with animal products such as eggs, poultry and dairy products, but also with plant origin food such as salad dressing, fruit juice and sesame. At the time of slaughter, *Salmonella*-infected poultry may have high numbers of organisms in their intestines as well as on the outside of the bird and are therefore an important source of contamination. Nowadays, food safety has become an important concern for the European society and governments; therefore, more strict and harmonized regulations are being implemented throughout the poultry production chain with the aim to guarantee and increase the consumer confidence in foodstuffs of animal origin. Furthermore, increasing antimicrobial resistance in non-typhoid *Salmonella* species has been a serious problem for public health worldwide.

Keywords: salmonellosis, foodborne, prevalence, poultry, antibiotic resistance

1. Introduction

Salmonella has been declared by the World Health Organization (WHO) and the Food Agriculture Organization (FAO) as the most common and important zoonosis since 1950.

This has led to its inclusion in the terrestrial animal health code of the World Animal Health Organization. In humans, typhoid disease manifests one to 2 weeks following bacterial inoculation with generalized fever and malaise, abdominal pain with or without other symptoms including headache, myalgias, nausea, anorexia and constipation [1]. An estimation of the annual non-typhoid *Salmonella* gastroenteritis suggests that there are around 94 million cases, resulting in 155,000 deaths, and that the majority of the disease burden, according to this study, is in the South-East Asian Region and the Western Pacific Region [2]. Most human salmonellosis cases are foodborne, but each year, infections are also acquired through direct or indirect animal contact in homes, veterinary clinics, zoos, farm environments or other public, professional or private settings. It has been estimated that approximately 80.3 million of 93.8 million human *Salmonella*-related gastroenteritis cases—that are diagnosed globally each year—are foodborne, thus representing approximately 86% of human salmonellosis cases [2]. Another study estimated that approximately 55% of human *Salmonella* cases were foodborne, 14% were travel-related, 13% are acquired through environmental sources, 9% occurred due to direct human-to-human transmission and 9% were attributable to direct animal contact [3, 4].

2. *Salmonella* species classification

The bacteria of the genus *Salmonella* are responsible for illnesses in human beings and animals. The genus is divided into two species: *Salmonella enterica* and *Salmonella bongori* [5]. *S. enterica* is divided into six subspecies (*enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*) and each one of them has several serovars or serotypes. Nowadays, more than 2500 serotypes are known and almost 1500 of them belong to the subspecies *enterica* [6]. Most pathogenic isolates from humans and other mammals belong to *S. enterica* subspecies *enterica*. Other *S. enterica* subspecies and *S. bongori* are more common in cold-blooded animals and the environment, with lower pathogenicity to humans and livestock [7, 8].

A few serotypes are host specific; i.e. *S. typhi* is implicated in typhoid fever in human beings, while *Salmonella pullorum* and *gallinarum* are responsible for bacillary white diarrhoea and fowl typhoid in poultry, respectively [9]. *Salmonella choleraesuis* is host restricted to pigs, *Salmonella* ser. *abortusovis* is involved in sheep abortions and *Salmonella dublin* infects bovines [10]. There are a number of non-host-specific serotypes that may infect several animal species, including humans, and these are generally responsible for foodborne diseases with foods of animal origin being the main source. From the early years, the most common agent of human foodborne disease was *Salmonella typhimurium*, but in the last few decades the frequency of *Salmonella enteritidis* has dramatically increased [11]. Almost 80 out of 2500 serovars are thought to be frequently involved in animal and human salmonellosis. *S. typhimurium* and *S. enteritidis* are the most common agents of disease in human beings and animals, but lately, there is also increasing concern about *S. typhimurium* monophasic, *S. derby*, *infantis*, *agona*, *hadar*, *heidelberg* and *virchow* serotypes.

3. Transmission routes, public health and economic cost associated with *Salmonella* infection

The gastrointestinal tracts of humans and animals are the primary sources of *Salmonella*. The bacteria are carried asymptotically in the intestines or gall bladder of many animals and are continuously or intermittently shed in the faeces. Also, these can be carried latently in the mesenteric lymph nodes or tonsils [12], which are not then shed, but can become reactivated after stress or immunosuppression [13]. Although most infections cause mild to moderate self-limiting disease, serious infections leading to deaths do occur [14]. Its widespread presence in the environment is considered to be due to the direct or indirect faecal contamination [15]. The transmission to humans usually occurs through the consumption of food or water contaminated with animal faeces, but it can also happen through direct contact with infected animals or their environment and directly between humans. In the same way, animals can become infected from contaminated feed (including pastures), drinking water or close contact with an infected animal (including humans).

Transovarian (vertical transmission) or trans-shell (horizontal transmission) occurs in poultry. In the first case, a contamination of the vitelline membrane, albumen and possibly the yolk of eggs occurs. Following this route, *Salmonellae* are introduced from infected reproductive tissues to eggs prior to shell formation. *Salmonella* serotypes with high importance to public health, associated with poultry reproductive tissues, include *S. enteritidis*, *S. typhimurium* and *Salmonella heidelberg*. Among all the different serotypes, *S. enteritidis* may be more invasive and, consequently, may be found more frequently in reproductive tissues. Faecal contamination of egg shell is the primary cause of horizontal transmission [16]. This can also include contamination through environmental vectors, such as farmers, pets and rodents, feed, water, fluff, dust, shavings and straw, insects, equipment, and thus, many different serotypes of the genus *Salmonella* can be involved [17, 18]. Bacteria can contaminate egg contents by migration through the egg shell and membranes. Such a route is facilitated by factors such as moist egg shells, storage at ambient temperature and shell damage. Faecal shedding of *S. enteritidis* was detected for up to 8-week post-inoculation by hens housed in enriched colony cages and 10 weeks by hens housed in conventional cages, which were experimentally infected with *S. enteritidis* [19]. Studies on the survival of *S. enteritidis* in poultry units and food were carried out over a 2-year period and showed that the organism persisted for at least 1 year in an empty trial house at the laboratory in which naturally infected broiler breeder birds had previously been housed [20]. In the same study, a similar survival period was found in a building which had housed an infected layer breeder flock, although infection was not detected in a subsequent pullet flock. *Salmonella* contamination appeared to persist preferentially in association with dust particles swept from the floor and in food troughs, and *S. enteritidis* survived at least 26 months in artificially contaminated poultry food [20].

Salmonella spp. can also be transmitted *in utero* in other mammals. Wild birds, rodents, fomites and mechanical vectors (insects) can spread *Salmonella* to livestock. In general, *Salmonella*

serotypes have a broad host range and clinical manifestations that result from the combination between serotype and host species involved [21] are prevalent in a whole range of warm-blooded animal population [22] but also in snakes [23], and free-living terrestrial and aquatic turtles [24].

Salmonella spp. can survive for long periods in the environment, particularly, where it is wet and warm. They can be isolated from many sources including farm effluents, human sewage and water. Persistence of *Salmonella* in acid soils is facilitated by their ability to adapt to low-pH environments [25]. There is also some evidence that *Salmonella* may survive in soils in a viable but non-culturable state [26], although significance of this state is not yet understood. *S. choleraesuis* has been isolated for up to 450 days from pig meat and for several months from faeces or faecal slurries [27]. *Salmonella typhimurium* and *Salmonella dublin* have been found for over a year in the environment.

Plant origin material can be contaminated through direct deposition of *Salmonella*-containing animal faeces or through deposition of soil or dust previously contaminated with animal faecal material. In some circumstances, there has been an increasing evidence that *Salmonella* may be internalized in plant tissues [28]. This, however, was quite uncertain whether it was relevant to crops commonly used as components of animal feed.

Person-to-person transmission of *Salmonella* is well-recognized, and secondary transmission of *Salmonella* in outbreaks has been demonstrated [29]. Carriage in faeces in convalescent cases can be quite substantial with numbers approximating 10^6 to 10^7 *Salmonella*/g persisting up to 10 days after initial diagnosis according to the authors. Reduction in numbers with time seems to be variable; most people will have count of less than 100 *Salmonella*/g after 35–40 days, but a count of 6×10^3 /g has been recorded in one patient 48 days post-illness [30]. Asymptomatic carriage may also occur, as it was mentioned for a British outbreak of hospital-acquired infection [31] and another case where asymptomatic food handlers have been responsible for an outbreak in a catering establishment in Jerusalem [32].

Non-typhoidal *Salmonella* are a leading cause of bacterial diarrhoea worldwide; they are estimated to cause 94 million cases of gastroenteritis and 115,000 deaths globally each year [2]. Of these, 80.3 million cases were estimated as foodborne origin. In one analysis [33] using data from the Foodborne Diseases Active Surveillance Network (FoodNet), the risk of *Salmonella* infection among travellers returning to the USA varied by region of the world visited. Travellers with salmonellosis were most likely to report visiting the following countries: Mexico (38% of travel-associated salmonellosis), India (9%), Jamaica (7%), the Dominican Republic (4%), China (3%) and the Bahamas (2%). Travel-associated infections were related to *Salmonella* in 36.7% of the cases reported, of which non-typhoidal *Salmonella* accounted for 88.3%, typhoidal *Salmonella* 7.7%, and paratyphoidal *Salmonella* 3.9%.

In the latest EFSA's report, a total of 82,694 confirmed salmonellosis cases were reported by 27 European Union (EU) member states in 2013, resulting in an EU notification rate of 20.4 cases per 100,000 population [11].

A decrease of 7.9% in the EU notification rate compared with 2012 was shown in the above report, which supports the declining trend of salmonellosis in the EU/European Economic

Area (EEA) in the 5-year period of 2009–2013 (**Figure 1**). However, the above was not statistically significant when analysed by month. Nine out of 14 EU member states reported a total of 59 fatal cases, which gave an EU case-fatality rate of 0.14% among the 40,976 confirmed cases. Some researchers claim that human salmonellosis represents a considerable economic impact and the estimated costing can be as €3 billion/year [34]. As in previous years, *S. enteritidis* and *S. typhimurium* represented 39.5 and 20.2%, respectively, in confirmed human cases, and they were the two most commonly reported *Salmonella* serovars in 2013 [11]. An interesting finding in the same report was that in the 2-year period from 2011 to 2013, cases of *S. typhimurium*, including the variant monophasic *S. typhimurium* 1,4,[5],12:i:-, decreased by 11.1%, while cases of *S. infantis* (which was the fourth most common serovar observed), increased by 26.5%. The fifth most common serovar observed in 2013, was *S. derby*, and this could partly be explained by a local outbreak in Berlin, Germany and surrounding areas in December 2013/January 2014. The outbreak occurred in hospitals and nursing homes with 145 elderly patients affected and one fatal case. The suspected vehicle of infection was rawfermented pork spread ('teewurst') [11].

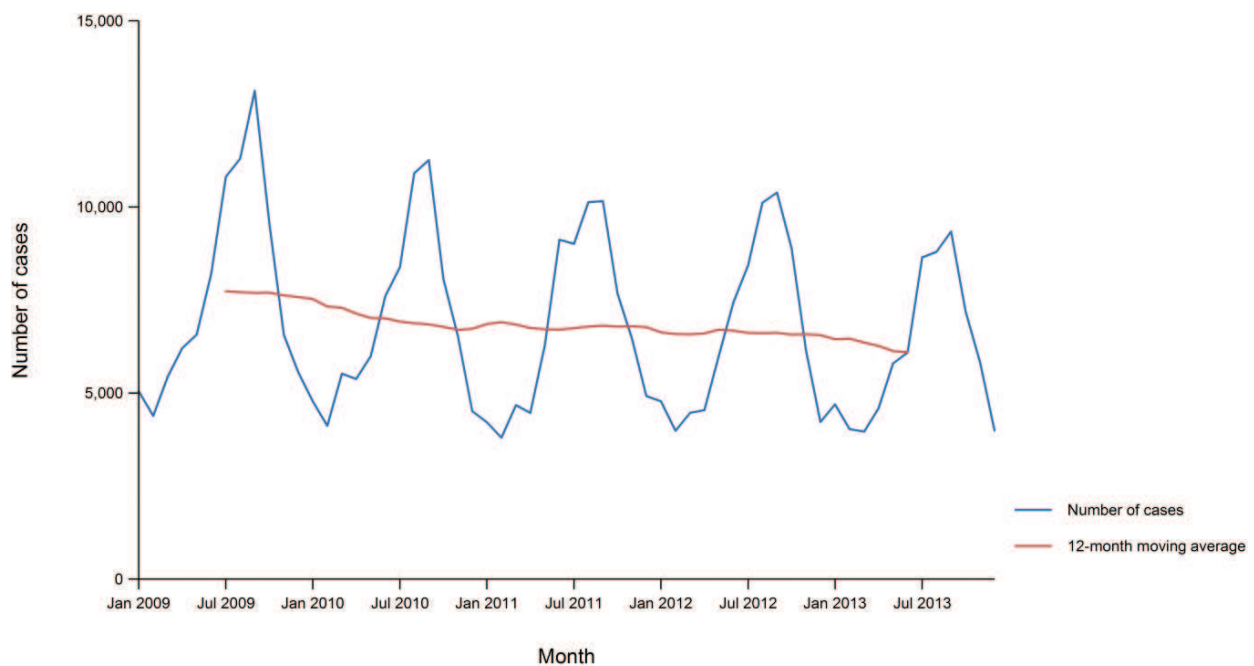


Figure 1. Trend in reported confirmed cases of human non-typhoidal salmonellosis in the EU/EEA, 2009–2013. Source: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Lithuania, Luxembourg, Malta, Netherlands, Norway, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden and United Kingdom. Bulgaria, Croatia, Italy, Latvia, Poland and Romania did not report data over the whole period in the level of detail needed for the analysis.

In a recent report published by USDA in 2015 [35], a comparison of the economic burden showed that *Salmonella* ranks first among the 15 pathogens included in the study and sixth on a per-case basis. It imposes an estimated \$3.7 billion in economic burden in a typical year. Almost 90% of this burden, thus \$3.3 billion, is due to deaths; 8%, \$294 million, is due to

hospitalization and the remaining 2% is due to non-hospitalized cases (hospitalization rate of 27.2% and a death rate of 0.5%).

According to Decision No. 2119/98/EC and 2000/96/EC, surveillance of foodborne salmonellosis in humans is mandatory in the EU member states as well as setting up a network for the epidemiological surveillance and control of communicable diseases in the Community [36, 37]. Data on humans, animals and food are compiled and analysed jointly by the European Food Safety Agency (EFSA) and the European Centre for Disease Prevention and Control (ECDC) and presented annually in the EU Summary Report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks [37].

4. *Salmonella* spp. in poultry and poultry products

Salmonella species that colonize the intestinal tract of poultry can persist throughout the bird's lifespan in a poultry-producing environment and are shed with faeces [9, 16]. Faecal shedding allows *Salmonella* to be transmitted among birds in a flock. *Salmonella* spp. is widespread in poultry production in Europe. Prevalence varies considerably depending on country and type of production as well as the detection methods applied. Results showed that prevalence is at the lowest level at the top of the production pyramid, i.e. the breeding stock. As mentioned above poultry meat and eggs represent an important source of human infection with *Salmonella* spp. with *S. enteritidis* and *S. typhimurium* been the most commonly reported serovars involved.

In the primary production, there are numerous activities that influence the introduction, growth or elimination of *Salmonella* species for poultry and poultry products, and therefore, many opportunities are given to *Salmonella* to enter the food chain, even though other steps will prevent growth or inactivate the pathogen. Several studies have studied the risk factors [38–40] associated with *Salmonella* contamination in broiler chickens. The most important risk factors included contaminated chicks, size of the farm (>3 poultry sheds—presumably related to increased human traffic among multiple sheds) and contaminated feed (the risk of *Salmonella* contamination of the flock was increased when feed trucks were parked near the entrance of the workers' change room and when feed meal, instead of small pellets). A systematic review of the risk factors associated with *Salmonella* in laying hens [41] concluded that the presence of previous *Salmonella* infection, absence of larger flock size (>30,000 hens), multi-age management, cage housing systems, rearing pullets on the floor, induced molting and in-line egg processing were factors associated with *Salmonella* infection. Also, cleaning and disinfection, presence of rodents, pests with access to feed prior to movement to the feed trough, visitors allowed in the layer houses and trucks near farms and air inlets were risks identified to be associated with *Salmonella* contamination of laying hen premises. However, high level of manure contamination, middle and late phase of production, high degree of egg-handling equipment contamination, flock size of >30,000, and egg production rate of >96% were identified as the risk factors associated with *Salmonella* contamination of shell eggs.

These were risks which showed strong to moderate evidence of association with *Salmonella* contamination of laying hens and shell eggs. In the same study, eggshells testing positive for *Salmonella* were 59 times higher when faecal samples were positive and nine times higher when floor dust samples were positive. Furthermore, the presence of *Salmonella enteritidis* infection in laying hens was associated with risk factors such as flock size, housing system and farms with hens of different ages.

The Panel on Biological Hazards [42] recommended that the application of hazard analysis critical control point (HACCP) principles, including good manufacturing practices and general hygiene procedures are recognized as important measures for *Salmonella* control in feed production. However, prevalence data for *Salmonella* in feed ingredients or compounded feed are usually very difficult to compare between different studies due to differences in sampling and analytical methods applied. The existing community legislation on food hygiene and control of zoonosis [43] constitutes a number of provisions that aim to control and prevent the *Salmonella* contamination of foodstuffs. Targets for *Salmonella* spp. were set progressively in different animal populations: breeding flocks of *Gallus gallus*, laying hens, broilers and turkeys. As an obligation, member states have developed and submitted national control programs to the commission which include recommendation on establishing strict biosecurity measures at farm level (including *Salmonella*-free poultry feed and water), vaccination programs in the parent flocks [44] as well as testing and removal of positive flocks from production. Except of encouraging immunity or resistance to *Salmonella* infection in birds through the use of antibodies, other strategies to prevent infection include the use of feed additives or acidified food. It is expected that acid treatments have a residual protective effect on feed, which reduces both the recontamination of feed as well as the contamination of milling and feeding equipment and the general environment [45]. However, the efficacy of organic acids against *Salmonella* depends on the level of bacterial contamination [46]. The same author recommends that, except feed treatment, water acidification can help prevent *Salmonella*, as the supplementation of acids in drinking water reduces the pH level and bacterial counts.

Nowadays, the trend seems to be towards production becoming more integrated, and many small farms will be replaced in the future by fewer, bigger farms, which will allow a greater integration and consequently to a better control of *Salmonella*. Furthermore, comparisons of *Salmonella* species contamination of free range or organic production systems with 'conventional' systems have produced varied results and more statistically valid surveys are required to ascertain if differences do occur [47]. In addition, the transportation of poultry between farms and from the farm to the processing plant offers an environment where *Salmonella* species might be spread between birds [48–50]. Shedding of large numbers of pathogens in faecal material during transport is believed to be related to increased stress in birds [48, 49].

Sewage and farm effluents, which can contaminate pasture, soil and water with *Salmonella*, tend to be handled more consciously lately, due to the pressure of environmental law requirements. However, the breeding stocks used all over the world are produced by a small number of companies, meaning that these sell to purchasers worldwide and this can lead to the

wide-scale spread of *Salmonella*, if the breeding stocks are infected. One should also take into consideration that where the aim is to control specific serotypes, a zero-tolerance policy with respect to these organisms may give a false sense of security, because the predominant serotypes in poultry flocks are likely to change over time.

5. Primary and secondary poultry processing and retail

The most important control measure at primary production, apart from those focusing in the elimination of *Salmonella* in grandparent and parent flocks by vaccination and an all-in-all-out production at the broiler farm, is to avoid any carry over during processing which is achieved by a logistic slaughter planning scheduled to avoid pathogens being transferred from contaminated processing equipment to another flock, and finally the satisfactory cleaning of transport crates. The operations that are thought to increase the contamination while in the processing line are scalding, plucking and evisceration. The most important critical control point in the process in relation to contamination is the feather plucker. Also, evisceration can be considered as an important risk due to a consequence of gut rupture. The evisceration machinery may play a role in damaging poultry carcasses while these are not entirely uniform in size. Most studies so far have shown that the prevalence of *Salmonella* species is usually higher on poultry carcasses at the end of primary processing than at the start [51, 52], although the concentrations of organisms on carcasses tend to decrease [17].

To reduce carcass contamination, decontamination measures can be applied. Many countries after the adoption of the 'Code of hygienic practice recommended for poultry processing' by the Codex Alimentarius in 1994, adopted their own code of practices for poultry processing. The requirements for cleaning of de-feathering equipment and recommended list of used disinfectants and practices of physical separation of de-feathering from later primary processing steps, requirements for processors to define acceptable levels of visible faecal contamination following evisceration and monitoring requirements for faecal contamination and practices of spaying or rinsing/dipping are included in this code. As far as these decontamination measures is concerned, one should take into consideration that, there are some regional differences, since chemical treatment is not accepted in the EU at the moment, but is widely used in other parts of the world, e.g. in the USA and New Zealand.

Poultry secondary processing includes portioning and processing of carcasses or portions into value-added products. During secondary processing, *Salmonella* prevalence may increase due to cross-contamination, while concentrations of *Salmonella* may increase if temperature control is not properly maintained [53]. Both poultry muscle and skin are excellent substrates for a wide variety of microorganisms [54], but the potential shelf life of raw poultry is quite short (e.g. chicken samples had spoiled after 4 days at 9°C) [55]. Unless frozen, raw poultry has a rapid turnover at retail, often 24–48 hours with a best before date of 3–4 days [56].

Salmonella species can survive well at refrigeration temperatures and will grow on fresh poultry under warmer, more favourable, temperatures (e.g. during transportation from a retail outlet to a consumer's home). *Salmonella* numbers are reduced under frozen storage conditions but salmonellosis outbreaks from 1998 to 2008 due to consumption of frozen

products showed that bacteria can survive freezing and *Salmonella* may pose an infection risk if the product is improperly cooked [57]. Thus, freezing cannot be considered as an adequate control step.

In a New Zealand consumer survey, the times and temperatures of purchased poultry products during transportation by consumers were examined [58]. It showed that thawing poultry at room temperature for up to 12 hours was a common practice and that any *Salmonella* present on the surface of the poultry could be able to grow once the surface reached room temperature [59]. Other studies have shown that the time required for frozen poultry (-18°C) to reach minimum growth temperature (7°C) would be in the range 3–16 hours, depending on the freezer temperature and ambient (air) temperatures [60]. As growth is greatly reduced up to 15°C (requiring another 3 hours thawing), and not optimal until $35\text{--}37^{\circ}\text{C}$, normal thawing periods before cooking are unlikely to permit much growth, although situations involving warm freezer temperatures (-7°C) and high ambient temperatures may increase the amount of growth that occurs.

The detection of *Salmonella* in poultry products leads to rejection of large consignments of raw poultry meat, thus affecting poultry trade with huge economic impacts as a consequence. Of course, on top of that, the impact on human health and the associated costs, the trade disruptions and the cost of implementing effective control measures explains why the Codex Alimentarius Commission (CAC) in 2010 [61] agreed that the development of guidelines for the control of *Salmonella* in poultry was a priority. Even though information on the prevalence of *Salmonella* on poultry meat at the end of processing or at retail were available, very few surveys have been undertaken where the number of organisms has been quantified [62] because enumeration of *Salmonella* proved to be very laborious.

Furthermore, interventions at the processing stages are assessed using growth models. These take into consideration several factors such as the levels of contamination when carcasses leave the processing plant, storage time in retail stores, transport time, storage times in homes and the temperatures carcasses were exposed to during each of these periods. It should be mentioned that the presence and level of *Salmonella* in this step is very much country specific, since the level of infection when leaving the processing step varies between the countries in relation to the methods which are used at the processing plant. In any case, national data must be used when estimating levels of contamination therefore [16].

6. Food of animal and plant origin as a source of *Salmonella* serovars for humans

Both plant and animal product-based animal feed ingredients may be contaminated with *Salmonella*. Red and white meat, meat products, milk, cheese and eggs are considered the major food sources of human salmonellosis, although a wide variety of other foods have been associated with outbreaks [8]. Other researchers reported that lamb's liver was responsible for an outbreak of *S. typhimurium* phage-type 197 in Australia [63]. In Germany, from 2001 to 2005, microbiological testing, trace-back investigations and epidemiological studies showed

that pork and pork products were involved in human salmonellosis outbreaks [64]. In Italy, an outbreak of *S. typhimurium* phage-type DT 104A involving 63 cases suggested that the consumption of pork salami was associated with this outbreak, underlining the importance of good manufacturing practices for ready-to-eat foods [65]. Many other reports involving human salmonellosis outbreaks associated with consumption of red meat have been recorded in the literature [66, 67], and in most of cases, the disease was associated with the consumption of contaminated meat or was a result of incorrect or inadequate cooking.

In the European Union (EU), contaminated foodstuffs serving as a source of *Salmonella* infection for humans include table eggs closely followed by pig meat, whereas the risks associated with broiler and turkey meat are similar and approximately two-fold lower [68]. As far as the distribution of serovars is concerned, in the EU, *S. enteritidis* and *S. typhimurium* are the serovars most commonly associated with human illness. Human cases of *S. enteritidis* are most frequently associated with the consumption of contaminated eggs and poultry meat, while *S. typhimurium* cases are associated with the consumption of contaminated pig meat or bovine meat [69]. It is estimated that around 10.6, 17, 56.8 and 2.6% of the human salmonellosis cases in the EU are attributable to broilers, laying hens (eggs), pigs and turkeys, respectively [70]. Of the broiler-associated human salmonellosis cases, around 82 and 6.5% are estimated to be due to the serovars *S. enteritidis* and *S. infantis*, respectively [71]. In the EU, approximately 9% of turkey carcasses are *Salmonella*-positive and the top six serovars that contribute to human cases are *S. enteritidis*, *S. kentucky*, *S. typhimurium*, *S. newport*, *S. virchow* and *S. saintpaul* [70]. While there are few data on the prevalence of pathogens on trimmings and meat cuts used for minced meat products, in [71] *Salmonella* spp. was detected on up to 5.3% of beef trimmings. The highest levels of non-compliance with *Salmonella* criteria generally occurred in foods of meat origin that are intended to be cooked before consumption in 2014, as in the last years [11]. Minced meat and meat preparations from poultry intended to be eaten cooked showed the highest level of non-compliance (category 1.5; 8.7% of single samples and 5.7% of batches). One should consider that growth of *Salmonella* should be absent or very slow in correctly chilled meat intended for preparation of mince since the organism show a reported minimum growth temperature of 5°C and an optimum temperature of 35–43°C [72], a pH growth range of 4.5–9.0.

A long list of foods that have been contaminated by *Salmonella* includes: seafood (shellfish, salmon), cereal and cereal products (barley, cereal powder), oilseeds and oilseed products (cottonseed, soybean sauce, sesame seeds), nuts and nut products (desiccated coconut, peanut butter), spices (white and black pepper, paprika), vegetables (watercress, tomatoes, lettuce, potato and other salads and bean sprouts), fruit and fruit products (watermelon, melon and cider) and other miscellaneous products (chocolate, cocoa powder, dried yeast and candy). *Salmonella* contaminated tahini (a product made from crushed sesame seeds) has caused a number of outbreaks worldwide, including New Zealand and Australia [73]. In 2002, an outbreak of *S. montevideo* occurred in New South Wales, Australia showing that imported 'tahini' was rapidly identified as the source of infection.

In foods from vegetable origin, detection of *Salmonella* serovars is a matter of increasing concern. Recent literature highlights the importance of foods of vegetable origin as potential vehicles of gastrointestinal infection nowadays. *Salmonella* serovars may contaminate vegetables during production, storage or even in retail outlets. Furthermore, fruits and juices, as they are usually consumed raw, may also be implicated in human salmonellosis.

In 2002, tomatoes, grown and packed in Virginia state (USA), contaminated with *S. newport*, caused illness in 510 patients in 26 other states [74]. Later, in July–November 2005, the same strain (confirmed by PFGE) caused illness in at least 72 patients in 16 states of the USA. *S. newport* strain was responsible for the outbreak which was isolated from pond water used to irrigate tomato fields, suggesting persistent contamination of the fields [75]. Also, during 2005–2006, in the USA and Canada three more outbreaks of *Salmonella* infections associated with eating tomatoes were detected. These outbreaks resulted in 387 culture-confirmed cases of salmonellosis, with isolation of *S. newport*, *S. braenderup* and *S. typhimurium* [76].

Unpasteurized orange juice was responsible for foodborne salmonellosis in 152 people in six states in the USA between May and July 2005 [77]. From 1995 to 2005, some researchers reviewed fruit juice-associated outbreaks of illness reported to Centres for Disease Control and Prevention (CDC), in Atlanta, USA [78]. Twenty-one juice-associated outbreaks were reported to CDC; 10 implicated apple juice or cider, eight were linked to orange juice and three implicated other types of fruit juice. These outbreaks caused 1366 illnesses, with an average of 21 cases per outbreak (range, 2–398 cases). Five out of 13 outbreaks of known aetiology, were caused by *Salmonella* serovars.

Human salmonellosis due to *S. thompson* infection were reported in Norway as a result of the consumption of rucola lettuce and mixed salad [79]. Prepared salads were also responsible for infectious intestinal disease outbreaks in England and Wales from 1992 to 2006 [80] as a result of international trade. Cross-contamination, infected food handler or inappropriate storage were the most common factors associated with this vegetable contamination.

7. Antibiotic resistance in *Salmonella* serovars: a serious problem in public health

Since 2003, according to the U.S. Food and Drug Administration, antimicrobial resistance in *Salmonella* spp., as well as in other bacterial species, has been recognized as a global threat and an increasing public health matter. Salmonellae have evolved not only virulence mechanisms to interact with host defence mechanisms at various tissues in different stages of infection resulting in significant host immunopathology, morbidity and mortality [1] but have evolved resistance mechanisms against antimicrobial agents, thus triggering host responses.

Individual organisms may transfer mutations that render antibiotics ineffective, passing on a survival advantage to the mutated strain, resulting in a normal genetic variation in bacterial populations. Advantageous mutations can also be conveyed via plasmid exchange within the bacterial colony, in the presence of antibiotics, resulting in proliferation of the resistance trait in the bacterial populations. Natural selection leads to an inherent consequence of exposure to antibiotic compounds and then antibiotic resistance arises.

On the other hand, the spread of particularly resistant clones and the occurrence of resistance genes within these clones can be exacerbated by the use of antimicrobials in human and animal populations and its selective pressure [81]. Many factors may also influence the spread of resistant clones, such as foreign travel by humans, international food trade, animal movements, farming systems, animal husbandry and the pyramidal structure of some

types of animal primary production. During the late 1990s and early 2000s, several clones of multi-drug-resistant (MDR) *Salmonella* emerged, and since then, they have expanded worldwide. Multi-drug-resistant *S. enterica* serotype *typhimurium* has been associated with a higher risk of invasive infection, higher frequency and duration of hospitalization, longer illness and increased risk of death as compared to infections caused by susceptible strains [82]. The spread of this resistance in other serotypes is of great concern as well. A very characteristic example is the behaviour of *S. typhimurium*, the genomic element that carries resistance to five antimicrobials (ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline), which can be spread horizontally among other serotypes and acquire additional resistance determinants. Genes conferring antimicrobial resistance in *Salmonella* are often carried on integrons and plasmids and could be transmitted through conjugation. These are mobile DNA elements and play an important role in transmission and dissemination of antimicrobial resistance determinants among *Salmonella* strains [83].

2013/652/EU Commission Decision sets an enhanced monitoring of antibiotic resistance (AMR) in bacteria from food and food-producing animals, which has been successfully implemented in all reporting and non-reporting member states. In accordance with the above legislation, the AMR monitoring started in 2014 and collected data referred on food and food-producing animals specifically targeted in different poultry populations and meat derived thereof. Two agents are responsible in performing the analyses of the data: EFSA and ECDC. The results are published in the first EU Summary Report on AMR [81] derived from 28 member states which reported data on AMR in zoonotic bacteria to the EFSA and 21 member states which submitted data to the ECDC. In the above report, the results showed that high proportions of human *Salmonella* isolates were resistant to tetracyclines (30.3%), sulphonamides (28.6%) and ampicillin (28.2%) and more than half (54.8%) of all isolates from humans were susceptible to the complete range of antimicrobial classes tested. A total of 8.8 and 1.1% of *Salmonella* isolates were resistant to ciprofloxacin to cefotaxime, respectively, which is thought to be an overall relatively low proportion of resistance to these clinically important antimicrobials. In the same report, resistance to third-generation cephalosporins was more common in *S. infantis* and *S. kentucky* with particularly high levels observed in Italy, most likely due to the circulation of a multiresistant and ESBL-producing (cefotaximase (CTX-M) type) clone of *S. infantis*. Also, an extremely high proportion (84.0%) of *S. kentucky* which showed high resistance to ciprofloxacin was mentioned. This is consistent with the dissemination of the ciprofloxacin-resistant *S. kentucky* ST198 strain in Europe and elsewhere since 2010 [84]. Overall, MDR in the EU was high (26.0%), with very high prevalence in some countries. It must be mentioned that some serovars exhibited very high to extremely high MDR. These were *S. kentucky* (74.6%), monophasic *S. typhimurium* 1,4,[5],12:i:- (69.4%) and *S. infantis* (61.9%). Another interesting observation derived from this study was the resistance to colistin which was commonly detected in *S. enteritidis* (67.5%, two member states) and it is thought that could be due to intrinsic resistance in this serovar.

In another study [85], it was reported that over 80% of strains from both human and animal sources that were tested for their antimicrobial resistance, showed that resistance patterns were similar among strains from humans and animals: the commonest phenotype comprised resistance to ampicillin, sulphonamides, streptomycin, chloramphenicol, and tetracycline and

was found in 76% of human and 73% of animal strains. Between 1972 and 1974, almost 50,000 *Salmonella* isolates from several sources (humans, animals, animal products, sewage, etc.) were tested for resistance to ampicillin, chloramphenicol, kanamycin and tetracycline in the Netherlands. The incidence of resistance to at least one of the above drugs ranged from 39.2 to 45.6%. An interesting finding was that multidrug-resistant strains of *S. typhimurium* and *S. dublin* were isolated from calves and cattle. A total of 64.4% of all strains of *S. typhimurium* from these animals appeared to be resistant to ampicillin, tetracycline, chloramphenicol and kanamycin, and 25.5% of *S. dublin* were found to be resistant to chloramphenicol and tetracycline in the latest year of the study [86]. In NARMS's last report which presents data for 2013 in the USA [87], *Salmonella*, antimicrobial resistance varies by serotype: 3% (61/2178) of non-typhoidal *Salmonella* isolates were resistant to nalidixic acid. The most common serotypes among the 55 ceftriaxone-resistant isolates were *S. newport*, *dublin*, *typhimurium*, *heidelberg* and *infantis*.

Overall, antimicrobial resistance varies among different serotypes of non-typhoidal *Salmonella*, and in some of them is considerably significant. It is well-recognized that the emergence of antimicrobial resistance in bacteria, which can be transferred to humans, is attributable to antimicrobial use in animals; therefore the effectiveness of antimicrobial drugs for treating human disease has been reduced extensively. The resistance to certain antimicrobials, especially fluoroquinolones and cephalosporins, are of particular concern with major consequences, since therapy of human systemic bacterial infections are critically dependent on their effectiveness. In face of this public health concern, it is highly recommended to follow a very careful prescription of antimicrobial agents during veterinary practice, regardless of the purpose of this prescription (prophylaxis or therapy) and a prudent use of antimicrobial agents after microbiological identification of the causative pathogen. Last but not least, it is very important to highlight that good hygiene practices and, wherever possible, alternative management methods should be sought and used and should not be substituted by the use of any antimicrobial agent.

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