

# ***Salmonella* in the pork production chain and its impact on human health in the European Union**

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## **SUMMARY**

*Salmonella* spp. comprise the second most common food-borne pathogens in the European Union (EU). The role of pigs as carriers of *Salmonella* has been intensively studied both on farm and at slaughter. *Salmonella* infection in pigs may cause fever, diarrhoea, prostration and mortality. However, most infected pigs remain healthy carriers, and those infected at the end of the fattening period could pose a threat to human health. Contamination of pig carcasses can occur on the slaughter line, and it is linked to cross-contamination from other carcasses and the presence of *Salmonella* in the environment. Therefore, *Salmonella* serovars present on pig carcasses can be different from those detected in the same batches on the farm. In recent years, *S. Typhimurium*, *S. Derby* and *S. serotype 4,[5],12:i:-* (a monophasic variant of *S. Typhimurium*) have been the most common serovars to be detected in pigs in EU countries, but *S. Rissen*, *S. Infantis*, *S. Enteritidis* and *S. Brandenburg* have also been reported. In humans, several cases of salmonellosis have been linked to the consumption of raw or undercooked pork and pork products. Among the main serovars of porcine origin detected in confirmed human cases, *S. Typhimurium*, the monophasic variant *S. 4,[5],12:i:-* and *S. Derby* are certainly the most important.

**Key words:** Humans, pig, pig carcass, *Salmonella enterica*, slaughterhouse.

## **INTRODUCTION**

*Salmonella enterica* is one of the most common and widely distributed food-borne pathogens in the European Union (EU) [1], and is considered one of the leading causes of gastroenteritis and bacteraemia in humans worldwide [2]. The most recent European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC)

report on zoonoses and zoonotic agents in the EU was published in 2015. This document presented the results of zoonoses monitoring activities carried out during 2014 in 32 European countries (28 member states and four non-member states). Human salmonellosis ranked second after campylobacteriosis with 88 715 confirmed cases and an EU notification rate of 23·4 cases per 100 000 population. After the declining trend observed in previous years, salmonellosis presented a 15·3% increase in the notification rate in 2014, compared with 2013 (20·3 cases per 100 000 population) [3].

Human salmonellosis is caused by both host-restricted (*S. Typhi* and *S. Paratyphi A*) and ubiquitous serovars,

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but only the former are responsible for the systemic life-threatening form of salmonellosis, referred to as typhoid fever [4]. Non-typhoid *Salmonella* infection is mainly characterised by gastroenteritis, with acute onset of fever, nausea, vomiting, abdominal cramps and diarrhoea; however, immunocompromised individuals may suffer from recurrent or prolonged *Salmonella* infections [5], whereas HIV patients and children could be affected by infections in the bloodstream, leading to death [6].

## **SALMONELLA IN THE PORK PRODUCTION CHAIN**

### **Salmonellosis in pigs**

Host adaptation of *Salmonella* serovars is of greatest importance in determining the clinical features and outcome of the infection. Apart from host-restricted serovars that are able to cause a typhoid-like disease in a single host species (e.g. *S. Typhi* and *S. Paratyphi A* in humans, and *S. Typhisuis* in pigs), other serovars, referred to as host-adapted serovars, are associated with one host species, but are also able to cause illness in other hosts (e.g. *S. Choleraesuis* in pigs and *S. Dublin* in cattle) [7]. *S. Choleraesuis* and *S. Dublin* generally cause severe systemic disease in pigs and cattle, respectively, but occasionally can be responsible for disease in other mammalian hosts, including humans [7]. In contrast, ubiquitous *Salmonella* serovars are the unrestricted serovars that are capable of causing systemic disease in a wide range of host animals, but more frequently cause a self-limiting gastroenteritis in a broad range of species. Examples of unrestricted serovars include *S. Typhimurium* and *S. Enteritidis* [7].

Infections with *S. Typhisuis* and *S. Choleraesuis* serovars in pigs usually result in swine paratyphoid, a severe systemic disease that is often fatal and characterised by fever, shivering, diarrhoea, respiratory distress and depression [8, 9]. Infected pigs exhibit clinical signs within 36–48 h after infection, and shed *S. Choleraesuis* in their faeces within 24 h of exposure. After experimental inoculation, shedding of the microorganism can vary between  $10^3$  and  $10^6$  colony-forming unit (CFU)/g of faeces. However, in natural infections, pigs might be exposed to lower levels of *S. Choleraesuis* (as low as  $4 \times 10^2$  CFU/g of faeces) that can still be responsible for high morbidity and severe outbreaks of swine paratyphoid within a relatively short period. Transmission of *S. Choleraesuis*

can thus be very efficient on the farm, during transport and in lairage before slaughter [9]. *S. Choleraesuis*, including variant Kunzendorf, was the predominant serovar isolated from pigs worldwide, over the past century (especially during the 1950s and 1960s). However, during the late 1990s and early 2000s, it was rarely detected in the EU countries [10, 11]. *S. Choleraesuis* var. Kunzendorf reappeared in Danish herds from 2012 to 2013, causing multiple outbreaks that were characterised by high mortality (20–30%) among 7–50 kg pigs [12].

Although infections in pigs by ubiquitous *Salmonella* serovars could result in enteric and even fatal disease, infected animals frequently and asymptotically carry these serovars in the tonsils, gut and gut-associated lymphoid tissue [10]. In EU countries, non-typhoidal *Salmonella* infections in pigs are related mostly to *S. Typhimurium* and its monophasic variant (antigenic formula 1,4,[5],12:i:-; 4,[5],12:i:-; or 4,12:i:-), *S. Derby* and *S. Infantis* [3, 13–15]. Other frequently reported serovars in pigs include Rissen, Panama, Goldcoast, Agona, Brandenburg, London, Anatum, Manhattan, Enteritidis and Bovismorbificans [3, 16, 17].

Transmission of *Salmonella* between pigs occurs mainly via the faecal–oral route [18], although some studies have demonstrated that the upper respiratory tract and lungs could be portals of entry as well [19]. A comparison between the prevalence of infection in pigs on the farm and in the abattoir revealed that prevalence on the farm often seems lower, partly because of the existence of latent, undetectable *Salmonella* carriers [20]. These latent carriers may begin to shed *Salmonella* only after leaving the farm, a process that might be triggered by stress factors linked to group housing, transportation and holding pens at the slaughterhouse, as the physiological changes associated with stress could promote recrudescence in latent carriers, or increase the susceptibility of non-carriers to new infections [21].

### ***Salmonella* in pig farms (pre-harvest stage)**

The ‘pre-harvest stage’ refers to that part of the food chain that includes the holding period of pigs on the farm until their departure and loading for transportation to the slaughterhouse [22]. During this period, pigs could become infected with *Salmonella* and show clinical signs, or they could become asymptomatic carriers and excrete the pathogen in faeces, or harbour it in several tissues, such as the digestive tract, closely associated lymph nodes or the tonsils [23].

Among the clinically affected pigs, most are of the weaning or post-weaning age, and the fattening pigs (body weight from 65 to 100 kg, and over) are generally asymptomatic carriers of unrestricted *Salmonella* serovars. The carrier status of pigs could be determined by the analysis of faecal cultures, obtained for example, via the collection of faeces with rectal swabs from randomly selected pigs during the fattening period [24]. The most frequently detected serovars in pigs on farms in the EU are *S. Typhimurium* monophasic variant, *S. Typhimurium* and *S. Derby* [13, 24, 25–27]. In 2014, the most common *Salmonella* serovars isolated from pigs in the EU were *S. Typhimurium* (50.3%), *S. Derby* (17.5%), *S. enterica* 1, 4,[5],12:i:- (8.4%), *S. Typhimurium* var. Copenhagen (4.4%), *S. London* (2.1%), *S. Infantis* (1.7%), *S. Muenchen* (1.6%), *S. Rissen* (1.5%), and *S. Livingstone* (1.2%) [3]. *S. Typhimurium* has been the predominant serotype detected within the last few years, accounting for as much as 72.8% of isolates in 2012. Since *S. Typhimurium* had been reported in 9 out of 10 member states in 2014, and was commonly reported in the baseline surveys of slaughter pigs and breeding pigs in 2006/2007 and 2009, respectively, its distribution across the EU can be assumed to be relatively wide [28, 29]. The prevalence of isolates of monophasic strains of *S. Typhimurium* has shown no considerable change over the last few years, ranging between 8.4% of all isolates in 2014, and 14% in 2013. Within recent years, Poland, Malta, the UK and Italy accounted for a large proportion of pig isolates of the monophasic *S. Typhimurium* (reported antigenic formulae 1,4,5,12:i:- and 1,4,1,2:i:-) [3].

With respect to the prevalence on positive farms, the EU baseline study performed in 2008 reported an average of 33.3% herds that tested positive for *Salmonella*, with a wide range among member states (0.0–55.7%). The most recent report on zoonoses and zoonotic agents in the EU, reported a 10.1% herd prevalence of *Salmonella* among nine countries in 2014 [3]. However, a comparison between the on-farm and abattoir prevalence of infection in pigs revealed that the on-farm prevalence is often underestimated. In Germany, the on-farm prevalence of infection in pigs, based on faecal analysis, was 5.58%; however, the caecal contents of 24.9% of slaughtered pigs were found to be *Salmonella* positive [24]. In a study by Hurd *et al.* [20], the abattoir prevalence of *Salmonella* in pigs was 39.9%, as opposed to an on-farm prevalence of 5.3%, a disparity that was probably due to the sample type (1 g faecal swabs on farm

vs. 10 g caecal contents after necropsy). The sensitivity of faecal samples collected on farm was particularly poor for pigs infected in the lymph nodes (0% on farm, 12.2% at abattoir). The sensitivity of faecal culture increases only twofold with a 10 g sample, as compared with a 1 g sample; thus, low faecal volume might partially explain the low sensitivity of samples collected from live pigs on farm [30]. Indeed, other factors might contribute to this discrepancy, such as the incidence of recent infections during transportation or slaughter [21]. Otherwise, the presence of latent undetectable carriers among infected pigs is a common characteristic in the epidemiology of *Salmonella* [20]. Moreover, intermittent shedding by pigs is a common feature that can interfere with monitoring and research programmes on *Salmonella* infection and the determination of health status in animals [31].

Prevalence can vary at the farm level, depending on various factors, such as feeding practices, including the degree to which the feed is ground, and the pH and type of feed; management procedures, such as continuous or all-in/all-out production system; different types of herds (farrow-to-finish herds or fattening herds); size of the herd; as well the level of hygiene and general health status of the pigs. The provision of safe feed is the first step in ensuring safe food, especially in a 'farm-to-fork' concept [32]. Therefore, pig feed should be *Salmonella*-free to guarantee a safer pork chain.

Wet feed has been demonstrated to reduce the risk of *Salmonella* infection, in comparison to pelleted feed, probably because of a fermentation step and consequent growth of lactic acid bacteria and yeasts [33]. The large amounts of organic acids produced, thus exert a protective effect, in a similar manner to the addition of organic acids to water [34] and feed [35]. In contrast, pelleted feed is considered one of the most consistently reported risk factors for *Salmonella* shedding in pigs [36]. In addition, feed particle size can affect the prevalence of *Salmonella* isolated from the gastrointestinal tract of pigs. In comparison to finely ground feed (<0.20–1.00 mm), coarsely ground feed (2.00–>3.15 mm) reduces the prevalence of *Salmonella*, by enhancing the fermentation of starch in the gut [37].

Regarding the influence of pH, values of 4.5 or lower effectively inhibit *Salmonella* both in the feed and in the gastrointestinal tract of pigs. Fermented liquid feed can yield such values, and improves the performance of sucklings, weaners and grower-finishers.

Feeding pigs fermented liquid feed prevents the growth and proliferation of pathogens, such as *Escherichia coli* and *Salmonella*, in the gut by reducing the pH in the stomach [38]. This is particularly important, because *Salmonella* has been detected in pig feed. In 2014, for example, some member states of the EU reported various serovars, including *S. Typhimurium*, *S. Enteritidis*, *S. Give*, *S. Agona*, *S. Anatum* and *S. Mbandaka* in the feed of pigs [3]. However, despite several studies on various interventions in feeding practices, there remains a lack of strong evidence of the effects between any association (acidification of liquid or pelletised feed vs. mash; coarse vs. finely ground feed; wet vs. dry feed, etc.) in reducing the prevalence of *Salmonella* on farm. Indeed, researchers do not consistently support the association between non-pelleted feed and a reduction in the prevalence of *Salmonella* in pigs [39].

The all-in/all-out management system reportedly has a protective effect against *Salmonella* infection [40], particularly when practised by the entire barn more than by single rooms [41]. The protective effect has also been observed when animals from different age groups are housed separately [42]. Contact with other species, as dogs and cats, can introduce the microorganism on farms, thereby increasing the risk of *Salmonella* infection [42]. Management practices also encompass the design of pen walls (solid, spindles or combination) and type of floor (fully slatted floors vs. <50% slatted floors can significantly reduce the prevalence of *Salmonella*) [13]. Nose-to-nose contact between pens is an important risk factor, as pens that allow direct contact among pigs are more likely to be *Salmonella* positive than those without such contact [36]. Poor pen cleaning and disinfection, and poor biosecurity measures are also important risk factors for the persistence of *Salmonella* at the farm level [43]. Cleaning by pressure washing with water, disinfection with chemicals and effective rodent control programmes are effective hygienic measures that should be adopted on farms [41]. Nevertheless, the resistance of *Salmonella* to some disinfectants, such as glutaraldehyde, formaldehyde and hydroxide peroxide at a concentration of 1.0%, make their use ineffective in field conditions, when it is protected by the development of a biofilm [44].

The prevalence of *Salmonella* shedding in pigs is higher in finishing farms (open farms) than in farrow-to-finish farms (closed farms) [45]. A Belgian study demonstrated that finishing farms were two times more likely to have *Salmonella* shedders than

farrow-to-finish farms, with positive rates of 10.3% and 5.4%, respectively (mean within-herd prevalence of 7.8%) [46]. Other authors have observed similar findings [45]. One possible explanation is that pigs raised in fattening farms originate from the piggery units of other farms. Upon arrival, they are frequently mixed with piglets of different origin, thus sharing the various health conditions of each (including *Salmonella* infection). Furthermore, transportation from piggeries to the finishing farms could be a stressful event that promotes the shedding of *Salmonella* by carrier animals, and the spread of infection throughout the barns [46].

In farrow-to-finish herds, where sows maintain the infection and excrete *Salmonella* particularly after weaning [47], prevalence might be influenced by herd size. Dors *et al.* [48] observed that prevalence in herds with more than 200 shedding sows was higher than in smaller herds. Furthermore, the size of finishing herds could increase the risk of development of the *Salmonella* carrier status in pigs, as this is usually higher in units that slaughter more than 3500 pigs per year [41].

### ***Salmonella* during transportation and holding (the harvest stage)**

The harvest stage refers to the part of the food chain that includes transportation of the animals from the farm, the lairage period, the slaughtering process and the cooling of carcasses [22].

During this stage, asymptomatic pigs could begin to shed *Salmonella* after having left the farm, owing to stress factors that are linked to group housing, transportation and holding at the slaughterhouse. Transportation significantly increases *Salmonella* shedding, thus, shedders become an important source of *Salmonella* to other pigs that are being transported [21, 49]. Stress can be caused by rough handling of the pigs at the time of loading and unloading, high stocking density during transport, long duration of transport, poor driver skills, adverse weather conditions and feed withdrawal. A relatively long feed withdrawal period, which is usually 12–18 h before transport, could be associated with changes in the gut microbiota, and elevated levels of *Salmonella* in the faeces [50].

Another factor that could influence the prevalence of infection among pigs at slaughter is the lairage duration. A positive relationship exists between the time spent at lairage and the frequency of *Salmonella*



detection in the lymph nodes, probably due to increased opportunity for invasion of the mesenteric lymph nodes (MLN) under conditions of prolonged stress. Pigs held for 12 h or more showed a greater chance of acquiring *Salmonella* from the lairage environment (16.7%), as compared with pigs held for 1–3 h (11.1%) [51].

Transportation and lairage conditions are thus important steps in the pork production chain that can increase the number of infected animals that are slaughtered. However, some studies suggest that external sources of infection might have a greater impact than stress, in increasing the detection of *Salmonella* at slaughter. For example, in a comparison of on-farm and slaughtered pigs, Hurd *et al.* [21] reported a seven-fold increase, and detected a variety of *Salmonella* serovars in necropsied animals, that had not been isolated from pigs on the farm. Such infections could be acquired before slaughter from various shedder pigs in transport trucks or the lairage environment, as infection of the gastrointestinal tract and infiltration of the associated lymph nodes can occur in as little as 2 h [52]. Rapid infection during transportation, and particularly during holding of pigs, is a major cause of increased *Salmonella* prevalence. Generally, the holding pen could be an important control point for *Salmonella* in the pork production chain [21]. Reduced exposure in trucks and holding pens is more likely to reduce *Salmonella* prevalence, than attempts to minimise stress, which is inevitable during transportation and lairage [53]. To facilitate the movement of pigs, stress at lairage can be kept under control by using well-designed infrastructure, well-lit corridors and minimal and careful handling of pigs, as specified by Regulation (EC) 1069/2009 [54], and discussed by some authors [55]. In addition, showering pigs when the temperature rises to >10°C [56] improves animal welfare at lairage. However, these measures can successfully reduce infections in pigs, only if the lairage environment is not already contaminated with *Salmonella*, thus posing a challenge for all slaughterhouses [55]. Contamination of the lairage might be responsible for oral infections in holding pigs, as well as skin contamination, which is directly related to carcass contamination during the slaughter process. In a comparison of carcasses with contaminated skin and those without, the probability of surface contamination of the carcass was reduced from 59% to 35%, respectively [57]. Furthermore, failure to dehair the carcass can significantly increase the number of contaminated carcasses [58].

### ***Salmonella* at slaughter (the harvest stage)**

The prevalence of *Salmonella* contamination in pork carcasses has been extensively studied in most European countries. Detection rates vary among studies, but all underline that *Salmonella* can be frequently isolated from MLN and faecal samples of pigs. Indeed, detection of *Salmonella* in the lymph nodes is frequently considered the ‘gold standard’ for definition of the carrier state at slaughter [22]. Alternatively, caecal material or faeces can be tested for carriage of *Salmonella* in pigs [22]. According to recent studies, the prevalence of *Salmonella* in MLN ranges from 7.4% to 26.0% [14, 24, 51, 59–62] in EU countries (Table 1). In 2014, very low levels of prevalence were detected in Sweden (0.0%) and Finland (0.03%), where control and eradication programmes reported the presence of *Salmonella* in the lymph nodes of finishing pigs [3]. Different surveys have reported prevalence at around 20–30% in faecal contents [11, 24, 63, 64] (Table 1). Furthermore, monitoring programmes based in the analysis of pig faeces in Denmark and Estonia reported rates of 21.6% and 27.3%, respectively [3]. Other data on the prevalence of *Salmonella* in pigs at slaughter are also available based on examination of the tonsils [24, 61, 63] (Table 1), mandibular lymph nodes [61], gall [24], heart and tongue [63].

Routes of contamination might be related to the pig or the slaughter environment. Contamination from the faeces of pigs that have been slaughtered on the same day might occur, with a typical distribution of *Salmonella* to the distal and medial surfaces of carcasses [65]. Contamination of carcasses with *Salmonella* on the skin of pigs has been demonstrated [58], but is probably less significant than faecal contamination [65]. In addition to the pig, the slaughter environment, in which microflora pose a potential risk for carcass contamination, is a major source of *Salmonella* [66]. Equipment, such as carcass splitters and belly openers, might be contaminated with *Salmonella* from fluids dripping from the carcasses onto the machines. Consequently, *Salmonella* on contaminated equipment could be transferred to other carcasses that are subsequently slaughtered [65]. *Salmonella* can also be spread by workers at the abattoir, as the hands of meat handlers can be frequently contaminated [59].

A marked reduction has been observed in the prevalence of *Salmonella* and the number of contaminated carcasses as the slaughtering process progresses

Table 1. *Prevalence of Salmonella in MLN, faeces, tonsils and carcasses of pigs at slaughter in different EU countries*

Prevalence (%)						
MLN	Faeces	Tonsils	Carcasses	Country	Years	Reference
26.0			16.0	Portugal	2007–2008	[58]
22.0	22.0		15.0	UK	2006–2007	[59]
19.9				Italy	2013–2014	[50]
18.8		9.9	12.9	Portugal	2003–2004	[60]
17.7				Belgium	2007	[61]
14.5	24.9	20.1	4.6	Germany	2005–2006	[24]
7.4			3.2	Denmark	2006–2007	[14]
0.03				Finland	2014	[3]
0.0				Sweden	2014	[3]
	30.5			UK	2013	[62]
	27.3			Estonia	2014	[3]
	23.0		5.3	UK	1999–2000	[11]
	21.6			Denmark	2014	[3]
	21.5	10.4	10.9	Italy	2005–2008	[63]
			5.0	Ireland	n.s.	[69]

EU, European Union; MLN, mesenteric lymph nodes; n.s., not shown.

[67], because of the steps taken to reduce bacterial flora on the skin of pigs. In one study examining a relatively large number of *Salmonella*-positive carcasses, the prevalence of *Salmonella* contamination was 96.6% at exsanguination and 35.9% after slaughter. During the slaughter process, skin contamination was reduced from 96.6% to 16.2%, but cross-contamination via equipment was responsible for the final number of *Salmonella*-positive carcasses reported [68]. Several authors have outlined that the main means of contamination is probably the result of a continuous cycle between pigs, the environment and the carcasses [62, 69].

Different levels of prevalence have been detected in pig carcasses in EU countries, ranging from 3.2% to 16% [11, 14, 59–61, 64, 70] (Table 1). Data from several countries in 2014, reported by the EFSA and ECDC are shown in Table 2. The differences observed could be attributed to several factors, such as the number of carrier pigs introduced to the slaughter line; implementation of effective steps for decontamination including dehairing, polishing and flaming; maintenance of good hygienic standards at slaughter; cross-contamination between carcasses and equipment; cross-contamination among carcasses; presence of resident slaughterhouse microflora and passive transmission via the hands of workers. Regulation (EC) 2015/1474 [71] regarding the use of recycled hot water to remove microbiological surface contamination

Table 2. *Prevalence of Salmonella in pig carcasses at slaughter in 2014*

Prevalence (%)	Number of tested animals	Country
12.75	447	Belgium
0.57	5392	Czech Republic
0.98	10 350	Denmark
3.12	385	Estonia
0.0	6398	Finland
0.35	1438	Hungary
0.44	450	Latvia
17.41	293	Spain
0.00	4921	Sweden
0.00	3025	Norway

Sampling unit: single pig [3].

from carcasses has recently offered increased opportunity to reduce the prevalence of *Salmonella*.

Because the pathogen is not only introduced to the slaughter line by the pigs, but could persist in the slaughterhouse environment, or be acquired during transportation and holding, the serovars isolated from on-farm samples can vary widely from those isolated after slaughter [24, 25, 49, 72]. The most commonly reported *Salmonella* serovars isolated from carcasses at slaughter are Derby, Typhimurium, Typhimurium monophasic variant, Rissen, Brandenburg, London, Manhattan, Muenchen and Stanley [51, 65]. The monophasic variant of *S. Typhimurium* (but not the biphasic *S. Typhimurium*) has been identified in pigs at slaughter

[51]. It is the most commonly isolated serovar in some countries, such as the UK, where it accounted for 32.9% of the serovars isolated from pigs in 2013 [73]. The rise in incidence of the monophasic variant of *S. Typhimurium* might be related to a novel clonal group that is characterised by the tetra-resistant pattern ASSuT (ampicillin, streptomycin, sulphonamides, tetracycline), which emerged during the 2000s in some EU countries, and has become particularly common in some member states, such as Italy, Denmark, the UK and Germany. In this clonal group, multidrug resistance is conferred by a new genomic island and the pattern ASSuT can be used for provisional identification of the isolates [74, 75].

### ***Salmonella* in the post-harvest stage: the EU Regulation (EC) No 2073/2005 criteria**

The criteria for *Salmonella* in foodstuffs, laid down by Regulation (EC) No 2073/2005 [76], have been in force since 1 January 2006. In member states of the EU, most national monitoring programmes for *Salmonella* in pork and pork products are based on the collection of swab samples of the carcass at the slaughterhouse, and/or meat samples at the processing plants. Regulation (EC) No 217/2014 [77] is a revision of Regulation 2073/2005 [76] and serves to reduce the acceptable number of *Salmonella*-positive pig carcasses from 5 out of 50 (10%) to 3 out of 50 (6%). Therefore, food business operators have to implement appropriate interventions to reduce the number of contaminated carcasses.

The studies on pork and ready-to-eat pork products have not been uniformly conducted among various EU countries, and show differences in sampling procedures, types of end-products and detection methods. Thus, consideration was given only to the most recent data provided by EFSA, which reported an overall *Salmonella* prevalence of 0.5% in fresh pork and 0.7% in ready-to-eat minced meat, meat preparations and meat products. Despite these relatively low numbers, pork and pork products, especially if consumed raw or undercooked, frequently represent a source of non-typhoidal *Salmonella* strains to humans [3].

### **PIGS AS A SOURCE OF SALMONELLA TO HUMANS**

Besides poultry, laying hens and turkeys, pigs are one of the major animal species that are responsible for the transmission of *Salmonella* to humans. However, their

role in food-borne salmonellosis in humans varies among EU countries. *Salmonella* source attribution studies estimate that pigs are a major source of salmonellosis in Southern Europe, accounting for 43.6% of all cases, whereas laying hens are the most significant source in Northern, Eastern and Western EU countries accounting for between 30.0% and 57.6% of all reported cases [1]. Overall, laying hens (via the eggs) represents the most important source of human salmonellosis in the EU, accounting for 42.4% of all cases, followed by pigs, accounting for 31.1%. Pigs are the major contributors of salmonellosis in eight countries, namely Belgium, Cyprus, Finland, France, Ireland, Italy, Poland and Sweden, whereas disease attribution to laying hens and pigs are similar in the Netherlands [78].

The role of pork in food-borne outbreaks of human salmonellosis has been demonstrated in several investigations, and many isolates detected in pigs have been responsible for human cases [51]. Overall, pork is ranked third among food categories that show strong epidemiological evidence of an association with human outbreaks of salmonellosis. The highest ranked food category is eggs and egg products, and the second, baked products, which were each responsible for 44.0% and 12.9% of outbreaks of human salmonellosis in 2014, respectively. Pork and pork products show strong evidence of an association with 9.3% of outbreaks reported in the EU. Pork is therefore the most significant source of meat that is responsible for the transmission of *Salmonella* to consumers [3].

Furthermore, when the source is known, the category 'pork and products thereof' is the mode of transmission most frequently associated with *S. Typhimurium* outbreaks [3]. Because *S. Typhimurium* and its monophasic variant are prevalent both on farm and at slaughter, isolation of this serovar in strong evidence outbreaks attributed to the consumption of pork is not surprising. In 2014, the most common *Salmonella* serovars isolated from pork and pork products in various EU countries were *S. Typhimurium* (28.3%), *S. Derby* (23.6%), *S. Typhimurium* monophasic variant (18.0%), *S. Infantis* (8.8%), *S. Rissen* (4.9%), *S. Brandenburg* (4.9%) and *S. Enteritidis* (2.1%). Although *S. Typhimurium* was the most commonly isolated serovar from both pigs and pork, the isolation of *S. Typhimurium* in pigs was significantly higher (54.7%) than it was in pork. This could be attributed to the fact that some countries submit more data on *Salmonella* from pigs than from pork, thus reducing the reported prevalence of *S. Typhimurium* in pork [3].

With respect to human salmonellosis, recent information on *Salmonella* serovars collected at the EU level, highlights the most common as *S. Enteritidis* and *S. Typhimurium*, which account for 44.4% and 17.4%, respectively, of all serovars reported in 2014. These serovars have been identified in all member states of the EU. The monophasic variant of *S. Typhimurium* represents the third most common serovar, responsible for 7.8% of all notified human cases. In order of frequency, *S. Infantis* (2.5%), *S. Stanley* (1%) and *S. Derby* (1%) are reported in fewer confirmed human cases, but are more widely distributed in several countries [3]. The pig-adapted *S. Choleraesuis* causes a serious infection in humans that is associated with high mortality, tends to be more invasive and cause fewer gastrointestinal symptoms than most other serovars [79]. Fortunately, it is not a common serovar in humans [3], despite the reappearance of the Kunzendorf variant within recent years [12].

European data on the most frequently isolated *Salmonella* serovars, confirm that they can all be detected in pigs and pork, but in varying proportions. Otherwise, to establish possible epidemiological correlations between porcine and human strains, genotyping of the isolates responsible for human cases that have also been detected in suspicious food sources, should be performed. *Salmonella* isolates can be subtyped by pulsed-field gel electrophoresis (PFGE), multiple-locus variable-number tandem-repeats analysis (MLVA) and patterns of antimicrobial resistance, in order to characterise the isolates that are associated with outbreaks. For example, in 2011, one major outbreak and several geographically dispersed smaller outbreaks that had been linked to pork were traced back to a butcher's shop and a pig farm in England, where a multidrug-resistant ASSuTTm (ampicillin, streptomycin, sulphonamides, tetracycline, trimethoprim) strain of *S. Typhimurium* phage type 120 (DT120) was isolated [80]. An outbreak at a wedding in Italy in 2011 was caused by the monophasic variant of *S. Typhimurium* 4,[5],12:i:-, of the rare phage type DT7a [81]. Since the source was identified in a cooked pork product, epidemiological investigations on the farm of origin revealed that the pigs carried a different serovar (biphasic *S. Typhimurium*) of the same phage type DT7a in their faeces. To identify specifically the most suitable subtyping methods by which the isolates associated with this outbreak could have been characterised, isolates from humans, pork and pigs were typed using *Xba*I PFGE, MLVA and patterns of

antimicrobial resistance. That study could not demonstrate whether isolates of the outbreak were directly related to isolates from the animals, but suggested that MLVA in particular, could be a reliable tool to support outbreak investigations and assess the genetic relatedness among *Salmonella* isolates [81]. In Italy two outbreaks of *S. 4,[5],12:i:-* DT193 were found to be caused by different strains, as the isolates were characterised using both *Bln*I-PFGE and MLVA [82]. In contrast, *Xba*I PFGE showed that the strains associated with the outbreaks were undistinguishable [82]. Characterisation of *Salmonella* is essential for proper identification, tracking and intervention during food-borne outbreaks. The phenotypic methods that traditionally provide important epidemiologic data during outbreak investigations have reduced value as typing tools for the surveillance and detection of common sources during outbreaks [81]. Within recent years, WGS (whole-genome sequencing) has increasingly become more readily available, and is routinely used as a powerful tool in diagnostic and epidemiological investigations during outbreaks and in various studies on infectious bacteria [83].

Molecular investigations and studies in antimicrobial resistance have been conducted both in food-borne outbreaks [81–83] and in research studies [51, 84–87], demonstrating the epidemiological connection between porcine and human compartments. In Italy, a comparison of *Xba*I PFGE profiles of porcine and human *Salmonella* isolates demonstrated shared profiles of *S. Derby*, *S. 4,[5],12:i:-*, *S. Rissen*, *S. Manhattan*, *S. Brandenburg*, *S. Livingstone*, *S. London* and *S. Muenchen* [51]. The relationship between porcine and human cases of salmonellosis (*S. Derby*) has also been demonstrated in France, in a study that typed porcine and human isolates with *Xba*I, *Bln*I and *Spe*I PFGE [84]. Most *S. Derby* isolates from pigs and humans were found to be resistant to streptomycin, sulphonamides and tetracycline (R-type SSuT) [84]. The whole-genome sequence of the most commonly detected strain of *S. Derby* in French pigs was recently characterised. The porcine isolate showed PFGE profiles and patterns of resistance (S, SSu, T) that have also been frequently identified in human isolates of *Salmonella* [84]. In Switzerland, two distinct clones of *S. 4,[5],12:i:-*, showing the ASSuT and SSuT patterns of antimicrobial resistance, were identified among human and porcine isolates [86].

Food-borne outbreaks of *S. Typhimurium* associated with pork products have been frequently reported, and



have been associated with the consumption of dried pork sausages in Spain [88], pork in England [80], smoked pork tenderloin [89], ready-to-eat spreadable pork sausage (Teewurst) [90] and salami produced with pork and venison in Denmark [91], and pork salami in Italy [92]. *S. Typhimurium* monophasic variant was identified as the causative agent of an outbreak in Germany following the consumption of minced pork [93] and in Italy following the consumption of cooked pork [81]. In another outbreak in Spain, both monophasic and biphasic *S. Typhimurium* strains, as well as *S. Derby*, were associated with the consumption of dried pork sausages [88].

*S. Derby* is strongly associated with pigs and pork products. In 2013, a food-borne outbreak of *S. Derby* affected 145 elderly patients and caused one death in Berlin, Germany, following the consumption of Teewurst [94]. Another outbreak was reported in France during the same year, and *S. Derby* was isolated from a typical meal, in which cross-contamination of the meat (beef and pork) probably occurred during preparation [73].

In 2014, Germany reported one food-borne outbreak of *S. Muenchen* that affected 164 people, of which four persons died. This outbreak was associated with the consumption of mostly raw pork products, in private households and a residential institution. A comprehensive investigation was conducted and the outbreak strain was detected in various food samples and in primary pig production facilities [3].

Over the last decade, Germany, Italy, the UK and Portugal have reported an increased prevalence of *S. Typhimurium* monophasic variant in pig populations [51, 74, 95–97], and consequently in humans affected by salmonellosis [3]. Another emerging serovar related to the pig is *S. Brandenburg* [51, 98], which has been increasingly isolated from humans affected by the disease [3, 51, 73]. Although it is not among the most frequently detected serovars in pigs in Europe generally, *S. Rissen* is common in pigs in Southern Europe [51, 59]. It is among the most frequently detected serovars in humans and pork production systems in several parts of the world, particularly Asia, and frequently detected in the USA [99]. Over the last few years, *S. Rissen* has been rarely detected in the EU, and the number of confirmed human cases is relatively low [3]. As Far-Eastern strains of epidemic multidrug-resistant *S. Rissen* have been isolated from pigs in some countries, for example Spain [100], further dissemination to other member states is possible.

## CONCLUSIONS

Salmonellosis has a major impact on human health, being the second most frequently reported zoonosis in EU countries [3]. Among food animals, pigs are estimated to be the second largest contributor to human cases of salmonellosis in the EU, after laying hens. For this reason, the symptomatic or asymptomatic carriage by pigs, epidemiology of the infection in herds, distribution of *Salmonella* serovars among pigs and contamination routes at slaughter have all been intensively investigated.

Many risk factors exist on the pig farm, including those related to feed, animal management, hygiene and biosecurity. The complexity of interactions among these factors can either amplify or reduce the prevalence *Salmonella* in pigs (Fig. 1). Consequently, implementing a unique strategy to reduce the levels of *Salmonella* in the pork production chain is a major challenge, especially as it relates to farm management, which over several years, can progressively change risk factor patterns [42]. Transportation practices and holding at slaughter are often responsible for contamination among animals, and these factors largely influence the prevalence of *Salmonella* in positive pigs entering the slaughter chain (Fig. 1). At slaughter, dehairing, polishing, flaming and sectioning operations can all affect the bacterial contamination of pork carcasses in several ways, and these processes generally do not include any hazard eliminating points [101]. Furthermore, one of the main risk factors for contamination is the persistence of *Salmonella* in the slaughter environment and the subsequent spread of variable serovars to pig carcasses. Nevertheless, many studies have demonstrated that good hygienic practices at slaughter are more effective in reducing the prevalence of *Salmonella* than on-farm interventions [102]. Abattoir interventions and their role in *Salmonella* control on pig carcasses are summarised in Figure 1.

Within recent years, *Salmonella* transmission from pigs to humans *via* the food chain has often been demonstrated, both in food-borne outbreaks and in epidemiological studies, with the aid of molecular techniques that are able to identify the strains responsible for porcine and human infections [51, 80–87]. The EU Regulation (EC) No. 2160/2003 [103] on zoonoses targets the reduction of *Salmonella* in animals and food products of animal origin during all phases of production, transformation and distribution, with an emphasis on primary production. Member states

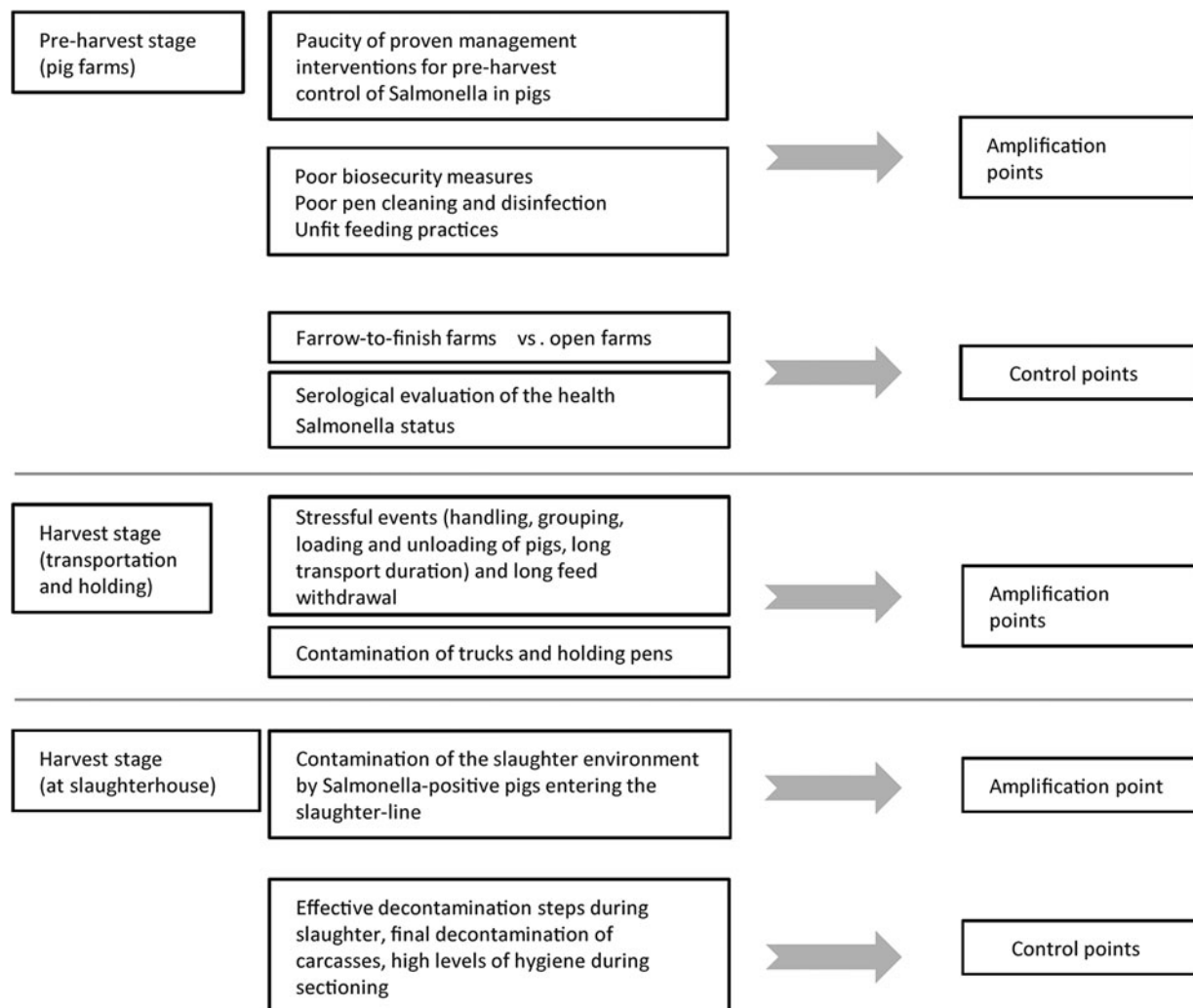


Fig. 1. *Salmonella* amplification and control points in the pork production system.

of the EU are required to take effective measures to control *Salmonella* in specific animal species (including pigs), and thereby lower the incidence of human salmonellosis. The interventions should be done on farm, at the slaughterhouse, or a combination of the two, as agreed by the member states [28]. Although quantitative microbiological risk assessment has shown that specific interventions at slaughter are more likely to produce a more significant reduction in cases of human illness than interventions at the level of primary production [104, 105], evaluation of the health status of pigs on the farm is still highly recommended. In some EU countries, such as Germany and Denmark, specific monitoring programmes categorise herds through a nationwide sampling scheme, based on *Salmonella* seroprevalence in pigs. Herds are categorised according to the

percentage of ELISA (enzyme-linked immunosorbent assay) seropositive samples present [106, 107]. Serological monitoring aims to estimate the risk for *Salmonella* at the level of the herd, and reduce the risk of introducing the pathogen into the meat production chain [108]. This type of monitoring should be considered a strategic tool for food safety in all European countries.

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## DECLARATION OF INTEREST

None.

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