Occurrence of *Salmonella* spp. in fattening pigs at a slaughterhouse in the Czech Republic

Helena Veselá[1], Marta Dušková[1], Petra Furmančíková[1], Renáta Karpíšková[1,2], Josef Kameník[1]

[1]University of Veterinary Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Department of Animal Origin Food and Gastronomic Sciences, Brno, Czech Republic
[2]Masaryk University, Faculty of Medicine, Department of Public Health, Brno, Czech Republic

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Abstract

The aim of this study was to evaluate the occurrence of *Salmonella* spp. in fattening pigs in a slaughterhouse in the Czech Republic. Tonsils, mesenteric lymph nodes, and caecal contents were sampled from 120 pigs originating from eight farms. A total of 360 samples were examined. Cultivation methods were used to detect *Salmonella*. Suspect isolates were subjected to phenotypic identification. Serotyping was performed by slide agglutination method. The multiplex polymerase chain reaction (PCR) was used to detect genes encoding flagellar proteins. *Salmonella* spp. was isolated in 3 out of 360 samples (0.83%). The results proved the presence of serovars *S.* Typhimurium, *S.* Derby, and monophasic *S.* Typhimurium. Although our study found a relatively low prevalence of *Salmonella* in fattening pigs at the Czech slaughterhouse, consistent implementation of *Salmonella* control program during pork production is critical in order to ensure the protection of consumer health.

Zoonosis, tonsils, lymph nodes, intestinal contents

In the second decade of the 21st century, bacteria of the genus *Salmonella* were the second most frequently reported zoonotic agent in humans in the EU (EFSA 2018; EFSA 2019; EFSA 2022). The most common causes of human salmonellosis are contaminated food (63%), followed by direct contact with animals (13%), mutual transmission (10%), and contaminated water (8%) (Christidis et al. 2020). The highest number of food samples positive for *Salmonella* can still be attributed to poultry production (eggs and meat), although in recent years, *Salmonella* frequency both in eggs and poultry meat has decreased significantly thanks to the control programs in EU Member States. Attention is therefore starting to be focused on pigs which are also involved in *Salmonella* spread. Besides poultry, pigs were the most frequently tested animals for this bacterium presence in 2021 (EFSA 2022). Pigs are most often infected on farms through various routes, including contaminated feed, water, or contact with other infected animals. In crowded and unsanitary conditions, the risk of *Salmonella* transmission can increase. Several studies also mention the important role of vectors such as insects, fleas, birds, cats, etc. (Vidic et al. 2015). *Salmonella* enters the oral cavity from the environment, where it colonizes the tonsils relatively fast (in approximately 30 min) and subsequently reach the lymph nodes via the lymphatic system. It is precisely these infected lymphatic tissues that may represent important reservoir of salmonellae (Vieira-Pinto et al. 2012). Pigs can be either carriers of *Salmonella* without showing any symptoms (asymptomatic carriers) or may exhibit clinical signs of infection such as diarrhoea, fever, loss of appetite, and lethargy. In severe cases, the infection can lead to dehydration and even death, especially in young or immunocompromised pigs.

Salmonella is relatively resistant in the farm environment. Faeces positive for *Salmonella* represent a significant risk for its spread. If *Salmonella* is introduced into the farm, the eradication of this pathogen is quite difficult (Rajtak et al. 2012). To reduce the risk

Address for correspondence:
Mgr. Marta Dušková, Ph.D.
University of Veterinary Sciences Brno
Faculty of Veterinary Hygiene and Ecology
Department of Animal Origin Food and Gastronomic Sciences
Palackého tř. 1946/1, 612 42 Brno, Czech Republic
Phone: +420 541 562 720
E-mail: duskovam@vfu.cz
http://actavet.vfu.cz/

of *Salmonella* transmission in pig populations and to ensure food safety, proper hygiene and biosecurity measures are essential on pig farms. Regular testing, monitoring, and control measures can help mitigate the spread of this pathogen in pigs.

Slaughterhouses represent one of the key places from which *Salmonella* can enter the food chain (Hdaifeh et al. 2020). The risk mainly consists in non-compliance with good hygienic and production practices, when bacteria are transferred from the animal's digestive tract to the meat during the slaughtering process. Another possibility is meat contamination from the surrounding environment. Fresh meat represents a favourable environment for the growth of *Salmonella* spp. due to high nutrient content, pH 5.5–6.5 and high water activity (aw = 0.98–0.99) (Chlebicz and Slizewska 2018). Consumption of pork is estimated to be the cause of human salmonellosis in roughly 15 to 23% cases in EU countries; however, these figures vary in different Member States. The highest confirmed human cases of salmonellosis in 2021 were reported by the Czech Republic (93.7 cases per 100,000 population) (EFSA 2022).

In 2019–2021, the most frequently confirmed serovars in human salmonellosis were *S.* Enteritidis, *S.* Typhimurium and monophasic *S.* Typhimurium (1,4,[5],12:i:-) (EFSA 2022). In pigs and pork, *S.* Typhimurium, monophasic *S.* Typhimurium (1,4,[5],12:i:-) and *S.* Derby were the most often isolated serovars in the EU (EFSA 2022).

In the Czech Republic, there is a lack of up-to-date complete studies that would deal with the issue of *Salmonella* occurrence in slaughtered pigs with the potential to contaminate meat at slaughterhouses (SVS 2020). A study carried out as a part of the EFSA project in 2006–2007 was focused only on the occurrence in mesenteric lymph nodes (EFSA 2008). The latest information was published as a part of the results of zoonoses monitoring carried out by the State Veterinary Administration. In addition to sponge sticks from carcasses, samples were newly taken from the caecum contents of fattening pigs (SVS 2021).

The aim of this study was to determine the presence of *Salmonella* spp. in lymphatic tissues and in caecal contents of pigs at the slaughterhouse in order to perform serotyping of obtained *Salmonella* isolates and to evaluate the transmission risk into the food chain.

Materials and Methods

Pig carcass sampling

For the purposes of this study, 120 fattening pigs slaughtered at a slaughterhouse in the Czech Republic between June and October in 2022 were tested for the presence of *Salmonella*. Tonsils, mesenteric lymph nodes and caecal contents were collected from each pig immediately after slaughter. The obtained samples were transported at the temperature of up to 4 °C and analysed within 2 h after collection.

Bacteriological analysis of the samples

To determine *Salmonella* spp., classical cultivation methods in compliance with ISO 6579-1:2017 (2017) were used. Samples in intestinal contents (25 g) were homogenized in 225 ml of buffered peptone water (OXOID, Basingstoke, UK); samples of tonsils (15 g) and mesenteric lymph nodes (15 g) were homogenized in 135 ml buffered peptone water. Samples were incubated at a temperature of 37 °C for 24 h. The homogenized intestinal contents were inoculated in three spots on Modified Semi-Solid Rappaport-Vassiliadis (MSRV) Agar (OXOID) with a total volume of 0.1 ml and incubated at a temperature of 41.5 °C for 24 h. Homogenized tonsil and lymph node samples of 1 ml volume were transferred to Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn; OXOID) and 0.1 ml volume to Rappaport Vassiliadis Soya Broth (RVS Broth; OXOID). Selective nutrient media were incubated at 37 and 41.5 °C for 24 h. Inoculation was performed on selective diagnostic solid media: Xylose Lysine Deoxycholate (XLD) Agar (OXOID) and Brilliance Salmonella Agar Base (OXOID), which were incubated at 37 °C for 24 h. The suspected colonies were subjected to serological (Salmonella latex test; OXOID) and biochemical confirmation (Enterotest 24; Erba Lachema, Brno, CZ).

Serotyping of *Salmonella* isolates

The typing was performed by the slide agglutination method with commercial antisera. *Salmonella* colonies were suspended in sterile physiological saline and evaluated for auto-agglutination prior to serotyping using 'O' and 'H' antisera. Colonies were serotyped by the slide agglutination method with commercial antisera (Denka Seiken, Tokyo, JP; BioRad Laboratories, Berkeley, USA) and the final antigenic structure was obtained according to the KauffmannWhite-Le Minor scheme (Grimont and Weill 2007).

The multiplex polymerase chain reaction (PCR) method using PPP Master Mix (Top Bio, Vestec, CZ) was used to detect evidence of genes encoding flagellar proteins. The PCR products were visualized by gel electrophoresis in a 2% agarose gel after DNA staining with intercalating dyes (McQuiston et al. 2004; EFSA 2010).

Results

A total of 360 samples were taken from 120 slaughtered pigs from eight farms in the Czech Republic. *Salmonella* was isolated from three pigs (2.5%). *Salmonella* was detected in lymphatic tissue samples (n = 3), specifically in tonsils (n = 1) and in mesenteric lymph nodes (n = 2). No *Salmonella* spp. was detected in the caecal contents (Table 1). A total of 0.83% of the tested samples were positive. A total of 3 different serovars were determined: *S*. Derby, *S*. Typhimurium and monophasic *S.* Typhimurium. The positive samples with the finding of *S.* Derby and *S*. Typhimurium came from the same pig farm, however, from different individuals. The monophasic *S*. Typhimurium came from a different pig farm.

Table 1. Serotype distribution of *Salmonella* isolates in samples from 3 positive pigs.

Serotype	Number of isolates per serotype		
	Tonsils (n = 120)	Lymph nodes (n = 120)	Intestinal contents (n = 120)
S. Typhimurium	-	1	-
Monophasic *S.* Typhimurium	-	1	-
S. Derby	1	-	-

Discussion

In positive pigs, *Salmonella* spp. is isolated mainly from tonsils, intestinal contents and mesenteric lymph nodes (Siddi et al. 2021). The results of our study showed the presence of *Salmonella* in 3 samples of lymphatic tissue, specifically in one sample of tonsils and in two samples of mesenteric lymph nodes. These results correspond with the results of other studies, where authors demonstrated the occurrence of *Salmonella* in lymph nodes (Bonardi 2017; Viana et al. 2019) and tonsils (De Busser et al. 2013; Van Damme et al. 2018). The isolation of *Salmonella* from mesenteric lymph nodes is associated with the long-term occurrence of *Salmonella* on farms; however, it has been confirmed that even short-term exposure during transport or in pre-slaughter housing can lead to lymph node positivity. The high prevalence in lymph nodes suggests that *Salmonella* remains a persistent problem on pig farms (Boughton et al. 2007; Deane et al. 2022). The fact that no *Salmonella* spp. was detected in the caecal contents samples in our study was surprising. These results are not consistent with previous studies where *Salmonella* was found to the largest extent in intestinal contents, particularly in rectal contents, ranging from 25.6% to 31% positive samples (De Busser et al. 2013; Van Damme et al. 2018), and in the caecum, ranging from 34.6% to 54.3% positive samples (Pesciaroli et al. 2017; Deane et al. 2022). A current study using *Salmonella* serotyping from the caecal contents of fattening pigs in the Czech Republic detected 6.5% positive samples, identifying the following serovars: *S.* Typhimurium (48%), *S.* Derby (29%), monophasic *S.* Typhimurium (4, [5],12:i:-) (9%), *S.* Enteritidis (9%) and *S*. Montevideo (5%) (SVS 2021). In 2020, the prevalence of *Salmonella spp*. in the carcasses of 4,562 pigs was 0.81% (SVS 2020).

Salmonella Typhimurium, *S*. Derby, and monophasic *S.* Typhimurium serovars have been most frequently associated with pigs in recent years (Pala et al. 2019). Further, the occurrence of serovars *S*. Rissen, *S*. Infantis, *S*. Enteritidis and *S*. Brandenburg was also recorded (Bonardi 2017). These serovars are commonly determined in the pig population in EU countries when the infection is subclinical. In 2021, most samples examined for the

presence of salmonella in EU countries, with the exception of poultry, came from pigs; in particular, *S.* Derby serovar was detected in 75.3% of samples where a pig was the primary source. For monophasic *S.* Typhimurium, 65.4% of positive samples came from pigs; for *S.* Typhimurium, 29.7% of samples were positive (EFSA 2022). We detected all three mentioned serovars in our samples. According to the latest studies, *S.* Derby poses a significant potential risk to human health if it enters the food chain. In case of *S.* Derby isolates, relatively long persistence in the environments of pig farms as well as biofilm formation were confirmed. In addition, resistance to a wide range of antiobiotics with different mechanisms of action was found (Simoni et al. 2022) as well as the ability to participate in the spread of antimicrobial resistance among animal species (Gonzalez-Santamarina et al. 2021). The monophasic *S.* Typhimurium serovar has been on the rise in the pig population in recent years and it is gradually replacing *S.* Typhimurium serovars (Bonardi et al. 2016; Cota et al. 2019). In 2017, the monophasic *S.* Typhimurium serovar was among the most frequently reported serovars occurring in pigs. Pigs currently represent the main reservoir of this serovar (Deane et al. 2022). Isolates are often resistant to numerous antimicrobial agents and heavy metals. For this reason they can pose a public health risk problem (EFSA 2010).

Slaughterhouses represent a critical site for spreading virulent and multi-resistant isolates of *Salmonella* spp. into the surrounding environment (Wu et al. 2021). Bacteriological examination of the pig organs at the time of slaughter is an important indicator in the risk assessment of carcass contamination. Carcass or meat contamination represent a key way for this pathogen to enter the food chain, thus posing a significant risk to food safety (Bonardi et al. 2016). The highest proportions of *Salmonella*-positive samples were determined on fattening pig farms, whereas the *Salmonella* prevalence in carcasses is much lower. Examination of sponge sticks from the carcass surface provides information on slaughter hygiene; examination of pig organs and intestinal contents is an important source of information on the *Salmonella* presence in pig farms. Reduction of *Salmonella* occurrence in pigs before slaughter, i.e., in the organs of slaughtered pigs, leads to fewer cases of carcass contamination, which reduces the risk of human infection due to the consumption of contaminated pork (Pesciaroli et al. 2017; Roasto et al. 2023). In order to prevent the *Salmonella* transmission to the food chain, it is important to observe veterinary measures in the breeding of slaughter animals; however, it is necessary to observe technological procedures and hygienic principles in food production as well. In some cases, *Salmonella* in pigs does not cause any clinical symptoms; this fact complicates significantly the chance to detect possible occurrence on farms. As a part of food businesses operation, there are procedures in place that are designed to prevent the introduction of infectious diseases. The measures are tailored to exactly suit precisely the facilities and operation of each manufacture's business. Common measures leading to the safety of meat produced at slaughterhouses include compliance with hygienic procedures during their production, feed supervision, proper waste disposal from carcasses, appropriately set cleaning and disinfection plans, etc. (Vidic et al. 2015).

The results of this study show low *Salmonella* prevalence on the tested farms. This finding is probably due to the implementation and adherence to the principles of good hygiene and production practice; regarding the *Salmonella* spread at slaughterhouses, it includes the slaughter of clean animals, prevention of carcass contamination with intestinal content, personnel training in terms of transmission methods and preventing carriers from entering the slaughterhouse area.

In conclusion, the study found a relatively low prevalence of *Salmonella* in fattening pigs at the Czech slaughterhouse, positive samples were detected only in the lymphatic tissue. This infected lymphatic tissue can serve as a source of contamination during the slaughtering process and therefore it poses a risk of introducing *Salmonella* into the food

chain. Serovars isolated from the samples in our research (*S.* Typhimurium, monophasic *S.* Typhimurium and *S.* Derby) are known to be associated with foodborne illnesses in humans, highlighting the importance of monitoring and controlling *Salmonella* contamination in the pig farming industry to ensure food safety and public health. Consistent implementation of *Salmonella* control program during pork production is critical in order to ensure the protection of consumer health.

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