

BEHAVIOR OF *SALMONELLA TYPHIMURIUM* IN PORK MINCED MEAT AND PORK SKIN AT DIFFERENT STORAGE TEMPERATURES

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Salmonella is a common contaminant of pork and can present a health hazard to consumers. Therefore, for an effective control, the entire supply chain must be involved. The aim of this paper is to examine the survival of *Salmonella Typhimurium* in pork minced meat and skin at different temperatures and time. In minced meat, stored at $+4 \pm 0.5^{\circ}\text{C}$ during 72 h, the number of *Salmonella Typhimurium* of all three examined strains A, B, and C decreased ($p < 0.01$). In minced meat, stored at $+10 \pm 1^{\circ}\text{C}$ after 72 h, the number of *Salmonella* strain A was significantly higher ($p < 0.01$), while for strains B and C, no statistically significant difference was determined ($p > 0.05$). On the skin, stored at $+4 \pm 0.5^{\circ}\text{C}$ during 72 h, the number of *Salmonella* of strains A and B decreased ($p < 0.01$), while for strain C, no statistically significant difference was determined ($p > 0.05$). On the skin, stored at $+10 \pm 1^{\circ}\text{C}$ during 72 h, the number of *Salmonella* of strains A and C decreased ($p < 0.01$), while for strain B, no statistically significant difference was determined ($p > 0.05$).

Key words: minced meat, pork, Salmonella, skin, temperature

INTRODUCTION

Salmonella can enter the food production process, e.g. in the primary stages by feed, people and rodents, during transport in contaminated lorries or at the slaughterhouses by cross contamination of animals and etc (Prendergast *et al.*, 2009; Karabasil *et al.*, 2008; Small *et al.*, 2002). This pathogen is a common contaminant of pork and can present a health hazard to consumers. Therefore, for an effective control, the entire supply chain must be involved. In Serbia, *Salmonella Enteritidis* is considered the most important foodborne *Salmonella*

causing illness in humans, followed by *Salmonella Typhimurium* (Institute of Public Health of Serbia and WHO, 2010). Pigs are an important reservoir of *Salmonella Typhimurium* (Delhalle *et al.*, 2009) and is the most frequent serotype isolated from porcine meat (Jordan *et al.*, 2006). The percentage and prevalence of *Salmonella* serotypes isolated from different surfaces in lairage, stunning boxes and from pork carcasses were as follows (Karabasil *et al.*, 2012c): *Salmonella Typhimurium* 68.6% (48/70), *Salmonella Mbandaka* 17.1% (12/70), *Salmonella* Senftenberg 8.6% (6/70), *Salmonella* Bredeney 4.3% (3/70) and *Salmonella* Menston 1.4% (1/70). Surfaces in pig lairages and in stunning boxes are regularly contaminated with *Salmonella* (Karabasil *et al.*, 2012a). *Salmonella* was isolated from 46.7% of examined carcasses immediately after stunning (Karabasil *et al.*, 2012b). The contamination rates for the different carcass areas were: brisket 30.0%, flank 23.3% and rump 13.3%. When contaminated carcasses are being processed, the main risk factors regarding cross contamination are inapt cleaning and disinfection, and manipulation of contaminated material (Janković *et al.*, 2012). Risk factors for human *Salmonella* infection include the consumption of contaminated meats, and handling of contaminated raw meat and cross-contamination with ready-to-eat products (Smerdon *et al.*, 2001). The aim of this paper is to examine the survival of *Salmonella Typhimurium* in pork minced meat and skin at different refrigeration temperatures and times.

MATERIAL AND METHODS

Microorganisms: *Salmonella Typhimurium* collections were made of two strains from slaughterhouse source: strain A and B (Faculty of Veterinary Medicine Belgrade); and one strain from clinical/human source: strain C (Public Health Institute of Serbia).

Samples for "in vitro" examination: Minced meat was prepared from pork ham (diameter grinders 6 mm) negative on *Salmonella*. pH of minced meat was 5.89. Prepared samples (50 g per sample) were contaminated with bacterial suspension of *Salmonella Typhimurium*, and left during 0, 24, 48 and 72 hours at $+4 \pm 0.5^{\circ}\text{C}$ and $+10 \pm 1^{\circ}\text{C}$. Parts of the skin (5 x 4 cm, from the flank), and previously tested and negative on *Salmonella*, were contaminated with bacterial suspension of *Salmonella Typhimurium*, and left during 0, 24, 48 and 72 hours at $+4 \pm 0.5^{\circ}\text{C}$ and $+10 \pm 1^{\circ}\text{C}$.

Bacteriological Analysis: "in vitro" studies were done, by contamination/inoculation of minced meat samples and skin samples, separate, with three strains A, B and C of *Salmonella Typhimurium*, in six replicates. The total number of *Salmonella* was determined from the serial dilution and 1 mL from each dilution was transferred in two Petri dishes and than poured with XLD agar, and incubated at 37°C for 24 h. The populations of bacterial cells were expressed in log CFU g⁻¹ (minced meat) and CFU cm⁻² (skin).

Statistical Analysis: Experiments were done in six replicates. Data were subjected to analysis using MS Office Excel 2003 and Statgraphics 5.0.

RESULTS

The bacterial suspension of *Salmonella Typhimurium* strains A, B and C was inoculated in previously prepared pork minced meat and skin samples, in order to determine the changes in the number of *Salmonella Typhimurium* under „*in vitro*“ conditions. The effect of temperature ($+4 \pm 0.5^{\circ}\text{C}$ and $+10 \pm 1^{\circ}\text{C}$) and time (0, 24, 48 and 72 h) on the changes in the population of *Salmonella* were studied.

Count of Salmonella Typhimurium in minced meat, stored at $+4 \pm 0.5^{\circ}\text{C}$

The average number of *Salmonella Typhimurium* strain A, B and C expressed in log CFU g^{-1} decreased after 72 h at $+4 \pm 0.5^{\circ}\text{C}$ compared with the initial value (0 h) (Table 1). On the basis of standard deviation (S_d) for the values before and after the effects of temperature/time, can be seen that the data for log CFU g^{-1} are homogeneous within each group. Since the coefficient of variation (C_v) is below 33% there was no significant variation in the bacterial population within the group. On the basis of the results of the LSD-test can be concluded that there is a significant difference in the change in the population of strains A, B and C before and after the treatment (Table 1).

Count of Salmonella Typhimurium in minced meat, stored at $+10 \pm 1^{\circ}\text{C}$

The average number of *Salmonella Typhimurium* strain A and B expressed in log CFU g^{-1} increased, while strain C practically remained unchanged, after 72 h at $+10 \pm 1^{\circ}\text{C}$ compared with the initial value (0 h) (Table 1). On the basis of the standard deviation (S_d) for the values before and after the effects of temperature / time it can be seen that data for log CFU g^{-1} are homogeneous within each group. Since the coefficient of variation (C_v) is below 33%, there was not a significant variation in the bacterial population within the group. On the basis of the LSD-test it can be concluded that there was a significant difference in the change in the population of strains A and B before and after the treatment (Table 1). Analysis of variance showed that the average number of strain C before and after the treatment was not statistically significant.

Count of Salmonella Typhimurium on pork skin, stored at $+4 \pm 0.5^{\circ}\text{C}$

The average number of *Salmonella Typhimurium* strains A and B expressed in log CFU cm^{-2} decreased, while strain C slightly decreased, after 72 h at $+4 \pm 0.5^{\circ}\text{C}$ compare with the initial value (0 h) (Table 2). On the basis of the standard deviation (S_d) for the values before and after the effects of temperature / time can be seen that the data for log CFU cm^{-2} are homogeneous within each group. Since the coefficient of variation (C_v) is below 33%, there was no significant variation in the population of bacteria within the group. On the basis of the results of the

LSD-test, can be concluded that there was a significant difference in the change in the population of strain A and B before and after the treatment (Table 2). Analysis of variance showed that the average number of strain C, before and after the treatment, was not statistically significant.

Table 1. Count of *Salmonella Typhimurium* in minced meat, stored at $+4 \pm 0.5^{\circ}\text{C}$ and $+10 \pm 1^{\circ}\text{C}$

Time	Temperature $+4 \pm 0.5^{\circ}\text{C}$						Temperature $+10 \pm 1^{\circ}\text{C}$					
	\bar{X}	S_d	S_e	X_{\max}	X_{\min}	C_v (%)	\bar{X}	S_d	S_e	X_{\max}	X_{\min}	C_v (%)
<i>Salmonella Typhimurium</i> strain A												
0 h	4.13 ^a	0.18	0.07	4.43	3.95	4.38	3.78 ^x	0.13	0.05	3.96	3.66	3.56
24 h	4.30 ^x	0.18	0.07	4.58	4.04	4.20	3.69 ^x	0.065	0.03	3.80	3.63	1.76
48 h	4.03	0.30	0.12	4.30	3.63	7.37	4.05 ^x	0.46	0.19	4.76	3.34	11.31
72 h	3.70 ^{b,y}	0.37	0.15	4.18	3.27	10.04	4.63 ^y	0.40	0.16	5.20	4.25	8.75
<i>Salmonella Typhimurium</i> strain B												
0 h	4.29 ^x	0.12	0.05	4.5	4.14	2.81	4.25 ^a	0.09	0.04	4.34	4.11	2.26
24 h	4.30 ^x	0.32	0.13	4.76	3.91	7.46	4.08 ^{b,x}	0.06	0.02	4.20	4.04	1.53
48 h	4.16 ^x	0.33	0.13	4.62	3.63	7.88	4.42 ^{b,y}	0.16	0.07	4.67	4.23	3.76
72 h	3.68 ^y	0.27	0.11	3.97	3.34	7.45	4.33 ^y	0.17	0.07	4.58	4.17	4.034
<i>Salmonella Typhimurium</i> strain C												
0 h	4.14 ^x	0.16	0.07	4.45	4.00	4.00	3.93	0.16	0.06	4.17	3.70	4.04
24 h	4.31 ^x	0.21	0.09	4.65	4.04	4.91	3.81	0.07	0.03	3.91	3.70	1.83
48 h	4.19 ^x	0.40	0.16	4.66	3.53	9.48	3.89	0.19	0.08	4.20	3.59	5.02
72 h	3.61 ^y	0.24	0.10	4.06	3.32	6.80	3.88	0.25	0.10	4.29	3.61	6.36

Legend: \bar{X} – mean value (log CFU g⁻¹, n=6); S_d – standard deviation; S_e – Standard error; X_{\max} – maximum; X_{\min} – minimum; C_v – coefficient of variation; a, b = p < 0.05; x, y = p < 0.01;

Count of *Salmonella Typhimurium* on pork skin, stored at $+10 \pm 1^{\circ}\text{C}$

The average number of *Salmonella Typhimurium* strain A, B and C expressed in log CFU cm⁻² decreased after 72 h at $+10 \pm 1^{\circ}\text{C}$ compared with the initial value (0 h) (Table 2). On the basis of the standard deviation (S_d) for the values before and after the effects of temperature / time can be seen that the data for log CFU cm⁻² are homogeneous within each group. Since the coefficient of variation (C_v) is below 33% there was no significant variation in the bacterial population within the group. On the basis of the results of the LSD-test can be concluded that there was a significant difference in the change in the population of strain A, B and C before and after the treatment (Table 2).

Table 2. Count of *Salmonella Typhimurium* on pork skin, stored at +4 ± 0.5°C and +10 ± 1°C

Time	Temperature + 4 ± 0.5°C						Temperature +10 ± 1°C					
	\bar{X}	S _d	S _e	X _{max}	X _{min}	C _v (%)	\bar{X}	S _d	S _e	X _{max}	X _{min}	C _v (%)
<i>Salmonella Typhimurium</i> strain A												
0 h	4.32 ^x	0.22	0.09	4.62	4.11	5.16	4.03 ^{a,x}	0.19	0.07	4.25	3.81	4.80
24 h	3.98 ^a	0.37	0.15	4.36	3.50	9.36	3.44 ^y	0.12	0.05	3.60	3.32	3.57
48 h	4.15 ^a	0.11	0.04	4.30	4.00	2.61	3.71 ^b	0.16	0.06	4.01	3.60	4.34
72 h	3.57 ^{b,y}	0.41	0.17	4.11	3.26	11.60	3.60 ^y	0.38	0.16	4.20	3.13	10.62
<i>Salmonella Typhimurium</i> strain B												
0 h	4.18 ^{a,x}	0.15	0.06	4.41	4.06	3.54	3.91 ^{a,x}	0.14	0.05	4.10	3.70	3.49
24 h	4.20 ^{a,x}	0.14	0.06	4.32	3.96	3.32	3.58 ^{b,c}	0.06	0.02	3.65	3.48	1.58
48 h	3.75 ^b	0.51	0.21	4.14	3.08	13.72	3.47 ^{c,y}	0.32	0.13	3.78	3.00	9.14
72 h	3.29 ^y	0.16	0.07	3.49	3.04	4.95	3.78 ^{a,b}	0.34	0.14	4.30	3.41	9.05
<i>Salmonella Typhimurium</i> strain C												
0 h	3.89	0.30	0.12	4.24	3.48	7.78	3.94 ^x	0.18	0.07	4.23	3.71	4.55
24 h	3.89	0.34	0.14	4.24	3.30	8.76	3.62 ^y	0.04	0.02	3.66	3.54	1.13
48 h	4.01	0.39	0.16	4.36	3.24	9.86	3.45 ^y	0.20	0.08	3.73	3.17	5.88
72 h	3.57	0.30	0.12	4.05	3.27	8.29	3.57 ^y	0.28	0.12	3.93	3.19	7.98

Legend: \bar{X} – mean value (log CFU cm⁻², n=6); S_d – standard deviation; S_e – Standard error; X_{max} – maximum; X_{min} – minimum; C_v – coefficient of variation; a, b, c = p < 0.05; x, y = p < 0.01;

DISCUSSION

Animals, feed, meat and its products are often transported long distances and represent a significant part of international trade, which enables the dissemination of *Salmonella*, including drug-resistant strains. Pigs are an important reservoir of *Salmonella*, which is “inside” the gastrointestinal tract and associated lymphoid tissue. On the other hand, in the last decade, it has been told much about the hypothesis that the food and feed chain are “too clean”. It was one of the reasons for some foodborne poisoning caused by bacteria (Dickinson and Olson, 2001; Jay, 1995). If this hypothesis is true, in future, we will face the problems related to subsequent contamination, and in the absence of competitive flora pathogens may be favored. In general, the safest way to control the growth of *Salmonella* is to maintain the cold chain and high temperatures. However, certain strains, like *Salmonella* Senftenberg are relatively resistant to heat. If the storage temperature is not below 7°C, *Salmonella* can multiply in the food. Also, it shouldn't be ignored that *Salmonella* is able to survive longer periods in foods where its multiplication for some reason is not possible.

According to the reported data, the most common *Salmonella* finding in pork is *Salmonella Typhimurium* (Delhalle *et al.*, 2009; Jordan *et al.*, 2006). Hence two strains of *Salmonella Typhimurium* (strain A and B) from a slaughterhouse source were chosen for the “*in vitro*” study. Besides these two strains, a third strain, from a clinical/human source, of *Salmonella Typhimurium* (strain C) was used, also. Samples of minced meat and pork skin, were checked on *Salmonella* presence and the results were negative. Contaminated samples, have been stored, in adequate cooling conditions at +4°C and in inadequate cooling conditions at +10°C during 0 h, 24 h, 48 h, and 72 h. According to the Regulations on the quality and other requirements for meat products (Official Gazette RS No 31/12), the prescribed temperature of minced meat at the moment of preparation and sale is 0 - 7°C and if prepacked is 0 - 2°C. It's not rare that in the retail network, prepared minced meat is kept in inadequate cooling conditions which often exceed +7°C, i.e. the critical temperature for growth of *Salmonella*. As well, consumers lifestyle has changed and once a week shopping is more common, leading to refrigeration of minced meat for hours or days before consumption (Koutsoumanis *et al.*, 2008). We wanted to determine whether there was a significant change in the number of these bacteria in pork minced meat under “*in vitro*” conditions, and if there is a difference between the strains isolated in the slaughterhouse and the clinical / human strain.

The finding of *Salmonella* in minced meat ranges from 3.2 to 57.1 % on the basis of 3237 samples examined in Mexico, Netherlands and the USA (D'Aoust, 2000). According to data (Moller *et al.*, 2013) when comparing growth curves of *Salmonella* obtained in sterile ground pork to curves obtained in pork with a natural microbiota at identical temperatures, they found that high concentrations of the natural microbiota reduce growth of *Salmonella*. The nature of the interaction was that growth of *Salmonella* in ground pork with a natural microbiota was identical to growth in sterile pork until high levels of the natural microbiota were reached. At that point, *Salmonella* growth slowed down considerably. We did not examine the interaction between natural microflora of minced meat and *Salmonella*. In the minced meat stored at +4 ± 0.5°C for 72 h, the number of all three investigated *Salmonella Typhimurium* strains A, B, C decreased, and a statistically significant difference ($p < 0.01$) between the initial number and the number of salmonella after 72 h of storage was determined. After 24 h of cooling there was no significant decrease in the number of strains A, B, C, nor after 48 h compared with the initial value of the number of salmonella strains. Under inadequate cooling conditions at +10 ± 1°C, after 72 h, the number of *Salmonella* strain A was significantly higher than the initial number ($p < 0.01$). The difference between the initial number of salmonella strains B and C and the number of salmonella strains in minced meat after 72 h of storage was not statistically significant ($p > 0.05$).

Previously published papers (Karabasil *et al.*, 2012a; Karabasil *et al.*, 2012b) was largely based on the pathways of contamination of pork carcasses in slaughterhouses. As already mentioned, the sources and pathways of

contamination of pork carcasses by *Salmonella* are numerous (Kranken *et al.*, 2003; Rostagno *et al.*, 2003; Hurd *et al.*, 2002). Therefore, it seemed interesting to see what happened with *Salmonella* on pork skin during refrigeration, whether the population decreased, stagnated or its number increased. The same refrigeration temperatures were applied for the the skin samples as for minced meat. Unlike minced meat, which contains fat, and can protect *Salmonella* from low temperatures, skin samples do not have such capabilities and its surface dries quickly, which is not favorable for *Salmonella*.

On the pork skin, after storing at $+4 \pm 0.5^{\circ}\text{C}$ during 72 h, the number of *Salmonella* strains A and B decreased, so there was a statistically significant difference between the initial number and the number of these *Salmonella* strains after 72 h of storage ($p < 0.01$). Between the initial number of *Salmonella* strain C and the number of the same *Salmonella* strain on the skin after 72 h of storage there was no statistically significant difference ($p > 0.05$). Numerically speaking, the number of *Salmonella* strains C, decreased compared to the initial value, but with no statistical significance after 72 h of storage. In inadequate storage conditions at $+10 \pm 1^{\circ}\text{C}$ during 72 h, the number of *Salmonella* strains A and C decreased, so that between the initial number and the number of these salmonella strains after 72 h of storage statistically significant difference ($p < 0.01$) was determined. Between the initial number of *Salmonella* strain B and the number of the same *Salmonella* strain on the skin after 72 h of storage there was no statistically significant difference ($p > 0.05$). The number of *Salmonella* strain C after 24 h ($p < 0.05$) and 48 h ($p < 0.01$) of storage was significantly decreased compared to the initial value. After 48 hours of storage of *Salmonella* strain B, the number of bacteria started to increase and after 24 h (72 h of storage), the number of bacteria was significantly higher ($p < 0.05$).

Salmonella, is often associated with outbreaks related to meat consumption and foodborne diseases worldwide (Rhoades JR *et al.*, 2009). Poor hygiene practice and improper food handling, might result in cross-contamination in meat processing plants (Perez-Rodriquez *et al.*, 2010) and in private kitchens too, even more frequently (Redmond and Griffith, 2003). Published results have showed that the tested equipment might contaminate food products during handling at various inoculum levels (Papadopoulou *et al.*, 2012.). Therefore, our results indicate the importance of using good hygiene and manufacturing practices in slaughterhouses and in households, as well.

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PREŽIVLJAVANJE *SALMONELLA TYPHIMURIUM* U USITNJENOM MESU I KOŽI SVINJA PRI RAZLIČITIM TEMPERATURAMA

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KURELJUŠIĆ JASNA, SOČO I. ĐURIĆ SPOMENKA i SAVIĆ RADOVANOVIĆ RADOSLAVA

SADRŽAJ

Salmonele predstavljaju važnije patogene, a meso svinja jedan je od glavnih izvora infekcije potrošača. Samim tim, za efektivnu kontrolu ovog patogena svaka karika u lancu hrane ima svoj značaj. Cilj ovog rada je bio da se ispita promena broja *Salmonella Typhimurium*, sojeva poreklom iz klanice i kliničkog/humanog materijala, u mlevenom mesu i koži svinja pri različitim temperaturama hlađenja i vremenu skladištenja, radi boljeg poznavanja pojedinih kritičnih mesta u lancu hrane. U mlevenom mesu, skladištenom pri $+4 \pm 0.5^{\circ}\text{C}$ u toku 72 h, broj salmonela sva tri ispitivana soja (*Salmonella Typhimurium* soj A, B, C) je opadao ($p < 0.01$). U mlevenom mesu skladištenom pri $+10 \pm 1^{\circ}\text{C}$ posle 72h broj salmonela soja A je značajno veći u odnosu na inicijalni broj ($p < 0.01$), dok kod sojeva B i C nije utvrđena statistički značajna razlika ($p > 0.05$). Na koži svinja, skladištenoj pri $+4 \pm 0.5^{\circ}\text{C}$ u toku 72 h, broj salmonela soja A odnosno B je opadao ($p < 0.01$), dok kod soja C nije utvrđena statistički značajna razlika ($p > 0.05$). Na koži svinja, skladištenoj pri $+10 \pm 1^{\circ}\text{C}$ u toku 72 h, broj salmonela soja A odnosno C je opadao ($p < 0.01$), dok kod soja B, nije utvrđena statistički značajna razlika ($p > 0.05$).