SALMONELLA IN WILD BOARS (*SUS SCROFA*): INFLUENCE OF HUNTING AND DRESSING PROCEDURES ON MEAT SAFETY

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Abstract

Salmonella spp. is considered as a high-priority foodborne hazard for control in wild boar meat. This comprehensive study about *Salmonella* spp. in population of wild boars in Serbia was conducted with an aim to assess the influence of hunting and dressing procedures on the spread of Salmonella on wild boar carcasses and to examine the molecular similarities of strains isolated from wild boars. Samples from wild boars from twelve hunting estates in South-West Vojvodina, Serbia, were taken from 425 hunted animals, which was 25.3% of the total wild boar population in all hunting estates. Three samples were taken from each animal's skin swabs, feces, and carcass meat swabs. A total of 1,275 samples were examined using standard ISO protocols. Subtyping of the isolates was performed and compared using Pulsed-field gel electrophoresis (PFGE). Salmonella prevalence was 4.2%, and *Salmonella Enteritidis* was the dominant serotype (74.5%). Carcass meat contamination originated from the feces of the same animal. Two or more entrance wounds, damage of the abdominal cavity caused by non-expert shooting were factors found to increase Salmonella contamination on the skin and/or on carcass meat. Rain during the hunting and practice of diaphragm and peritoneum removal and the evisceration being performed on the ground/floor or in hanging position did not lead to increase in Salmonella contamination on the meat. Although the determined prevalence was not high in wild boars compared to farm pigs, it is possible that *Salmonella* entered the food chain through contaminated meat. Therefore, the

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importance of good hunting and hygiene practice in handling and dressing wild boar carcasses should not be underestimated.

Keywords: wild boar, *Salmonella*, process hygiene, game meat, hunting procedures

SALMONELLA KOD DIVLJIH SVINJA (*SUS SCROFA*): UTICAJ PROCEDURA LOVA I OBRADE NA BEZBEDNOST MESA

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Kratak sadržaj

Smatra se da je *Salmonella* spp. visokoprioritetni patogen u kontroli mesa divljih svinja. U ovom radu su prikazani rezultati obimnog istraživanje Salmonella spp. u populaciji divljih svinja u Srbiji koja su sprovedena sa ciljem da se proceni uticaj procedura lova i obrade na širenje *Salmonella* na trupovima divljih svinja kao i da se utvrde molekularne sličnosti izolovanih sojeva. Uzorci su uzeti iz 12 lovišta sa područja Jugoistočne Vojvodine u Srbiji, ukupno je uzorkovano 425 ulovljenih životinja koje su u tom momentu činile 25.3% ukupne populacije divljih svinja u lovištima. Sa svake životinje su uzorkovana tri uzorka, bris kože, feces i bris trupa. Ukupno je ispitano 1,275 uzoraka standardnim ISO protokolom. Subtipizacije je izvršena primenom elektroforeze u pulsnom polju (PFGE). Utvrđena je prevalenca *Salmonella* od 4.2%, dok je *Salmonella Enteritidis* je bila dominantni serotip (74.5%). Takođe, potvrđeno je da kontaminacija trupa potiče iz fecesa iste životinje. Dve ili više ulaznih rana i oštećenje trbušne duplje uzrokovano ne-ekspertskim pucanjem su faktori koji povećavaju kontaminaciju kože i trupa sa *Salmonella* spp. Kiša tokom lova, praksa odstranjivanja dijafragme i periotneuma i evisceracija na terenu/podu ili u visećem položaju nisu doveli do rasta kontaminacije trupova sa *Salmonella* spp. Iako utvrđena prevalenca kod divljih svinja nije velika u odnosu na farmski uzgajane svinje i dalje postoji mogućnost ulaska Salmonella u lanac hrane preko kontaminiranog mesa. Stoga ne treba potceniti značaj dobre lovne i

dobre higijenske prakse prilikom rukovanja i obrade trupova divljih svinja.

Ključne reči: divlje svinje, *Salmonella*, higijena procesa, meso divljači, lovne procedure

INTRODUCTION

The European wild boar (*Sus scrofa*) is widely distributed in Europe, with a consistently increasing population since the second half of the last century (Massei et al., 2015). The biggest amount of game meat is consumed by hunters and their families, up to 1-4 kg/year per capita (Ramanzin et al., 2010), while its consumption is limited in the general population, 0.6-1.0 kg/year per capita in Austria, France, Germany and Switzerland (Atanassova et al., 2008; Membré et al., 2011). Consumers tend to follow trends of eating healthily (lower percentage of fat) and game meat is considered as a completely "natural" product because animals are raised without intensive farming.

Game meat harvesting and processing differ significantly from classical livestock meat production and represents a challenge itself. Domestic animals raised for food production in farm conditions are subject to regular veterinary health control and official *ante* and *post mortem* inspection at slaughterhouses, while in game species only *post mortem* examination is performed (Mirčeta et al., 2017). The game meat safety assurance and implementation of the concept "from forest to fork" encompasses the following: the influence of hunting estate environment, hunting and carcass dressing methods (including evisceration technique used), meat inspection after shooting, transport to the dressing/chilling facilities, etc (Petrović et al., 2014; Rodas et al., 2014; Mirčeta et al., 2017).

Salmonella frequently occurs in various types of meat used for human consumption and it is the most important pork meat pathogen in industrialized countries (EFSA, 2010). Wildlife could be transmission and accumulation vector of *Salmonella* in contact with domestic animals, direct contact with humans and through meat of water birds and wild boars (Hilbert et al, 2012). To the best of our knowledge, no outbreak of salmonellosis has ever been traced to the consumption of wild boar meat. This might be due to the general low consumption of game meat and/or the low incidence of *Salmonella* in wild boar meat (Hilbert et al., 2012). It is considered that *Salmonella* is a relevant biological hazard for hunted wild game animals, despite the fact that it is not considered a priority (Gortázar et al., 2007). It is well known that some of the hunting procedures for wild animals (such as skinning and carcass washing) ultimately lead to an increase in contamination of the carcasses (Mirčeta et

al., 2017; Orsoni et al., 2020). Information on the influence of the hunting and dressing processes on *Salmonella* presence on wild boar carcass meat from Serbia is lacking.

A large study was conducted on the *Salmonella* in wild boars, with the first part investigating its epidemiology, presence and distribution (Petrović et al., 2022). The aim of this second study was to assess the influence of the hunting and dressing process on the presence of *Salmonella* in wild boars. Molecular technique (PFGE) was used to determine the source of *Salmonella* carcass contamination by examination of molecular similarities of strains isolated from different sampling sites of wild boars.

MATERIAL AND METHODS

Study area and animals

Wild boars that originated from twelve different hunting estates in the region of South-West Vojvodina were examined in this study. Ten hunting estates investigated were large, fenced hunting estates, while two were open areas. Intensive management of wild boars for hunting purposes was used in all twelve hunting estates. Intensive management in Vojvodina encompasses habitat management to preserve the natural ecosystem, continuous monitoring of health status, sampling of hunted and dead wild boars supplementary feeding, and control of predators. The samples were collected during two hunting seasons (2013-2014) as part of a large project on pathogens occurring in wild boars. Research on the prevalence of *Salmonella* was supposed to continue but had to be stopped due to the outbreak of African swine fever (Polaček et al. 2021). The number of wild boars per hunting estate varied throughout the season, but it was estimated to be 1,677, out of which 425 were sampled. This was a representative sample, with 25.3% of the total wild boar population that was present at the moment of hunting when sampling was conducted.

Hunting and sampling procedures

The hunts were performed by using "still hunting method" (fixed positions for shooting) and using rifle bullets. At the end of the hunt, animals were usually collected and eviscerated either in the field at the collection point or transported to a respective game handling establishment for evisceration and dressing (not more than 2 hours). The sampling was conducted in winter hunting seasons. The fecal samples were taken directly from the rectum, approximately 50 g. Before sampling, sponge –swabs were moisturized with

Maximum Recovery Diluent (10 ml). Swab samples were taken by swabbing skin or carcass meat surface using the sponge-swab technique (Nasco Whirl-Pak™ Speci-Sponge). Skin swab samples were collected immediately before evisceration from approximately 1000 cm^2 of skin (lateral rump-perianal-medial rump-flank-brisket-neck). Carcass meat surface samples were taken shortly after the evisceration, and all other procedures were completed in no more than 10 minutes. Four carcass meat sites corresponding to the previously sampled skin (i.e. inner side of the rump and flank, thorax and brisket) were sponge-swabbed using sterile square plastic templates, which delineated a 100 cm^2 area (in total, 400 cm^2). In this study, samples from a total of 425 freshly shot wild boars were collected, with three samples from each animal tested for the presence of *Salmonella* spp.: skin swabs, feces, and carcass meat swabs (1,275 samples in total). All samples were transported in a chill-bin with external cooling system at 4°C to the laboratory within 3 h.

Microbiological procedure

The samples were stored at 4 ^{0}C and analyses were performed within 24 hours. Isolation of *Salmonella* was performed according to the ISO 6579:2002 sponge-swab samples and fecal samples procedures (Annex D) (ISO, 2002). Producers of all bacteriological culture media were Biokar Diagnostics, France and Oxoid, Ireland, except for Salmonella differential agar (Hi media, India). Suspected colonies of *Salmonella* spp. were further confirmed using API 20 *Enterobacteriaceae* (API 20E) strips (bioMérieux, Marcy l'Etoile, France). Serotyping was performed according to the CEN ISO/TR 6579-3:2014 (ISO, 2014) with commercial antisera (Institute of Public Health of Serbia "Dr Milan Jovanović Batut", Belgrade).

Characterization of isolated Salmonella strains

In order to investigate the possible source of *Salmonella* contamination isolates from different sampling sites of wild boars (n = 20; feces = 13, carcass = 5, skin = 2 – Table 1), subtyping of the isolates was performed and compared by pulsed-field gel electrophoresis (PFGE). *Salmonella* isolates were genotyped by applying the Standardized Laboratory Protocol for Molecular Subtyping of *Salmonella* by PFGE method (CDC, 2013). The macro-restriction of the genomic DNA was done with the S*peI* and X*baI* restriction enzymes. The macro-restriction of the *Salmonella* Braenderup H9812 strain was used as a molecular size standard. The obtained profiles were statistically analyzed by using Ward's linkage of correlation coefficients between PFGE patterns of different

genotypes using the SPSS cluster analysis software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). Hierarchical cluster analysis was performed in order to group serotypes with similar PFGE patterns resulting in a dendrogram (Zou et al., 2010). Length of the lines between serotypes shows similarities between serotypes. The shorter the length, the more similar the serotypes are. Distances between serotypes (from 0 to 25) reflect differences between clusters.

Table 1: List of isolates examined by PFGE

Isolate Sign	Sampling site	Hunting estate	Group	*Salmonella*
312	Feces	C	B	S. Typhimurium
313	Skin	E	B	S. Typhimurium
320	Feces	I	B	S. Typhimurium
321	Carcass	I	B	S. Typhimurium
344	Feces	D	B	S. Typhimurium
346	Feces	D	B	S. Typhimurium
317	Feces	G	D	S. Enteritidis
319	Carcass	I	D	S. Enteritidis
333	Feces	D	D	S. Enteritidis
336	Feces	D	D	S. Enteritidis
354	Feces	D	D	S. Enteritidis
355	Feces	D	D	S. Enteritidis
358	Feces	D	D	S. Enteritidis
375	Feces	E	D	S. Enteritidis
376	Feces	E	D	S. Enteritidis
246	Carcass	K	D	S. Enteritidis
248	Carcass	K	D	S. Enteritidis
249	Carcass	K	D	S. Enteritidis
254	Skin	K	D	S. Enteritidis
318	Feces	E	C	S. Infantis

Data analyses

The prevalence of *Salmonella*, χ2 square test and p value were calculated using IBM SPSS Statistics 20 (IBM, Armonk, NY, USA). The results of the

statistical tests were considered significant at p<0.05. Different hunting and dressing procedures that may have an impact on *Salmonella* prevalence were investigated. These included the following: 1. rainfall during the hunting, 2. the number of entrance wounds, 3. damage to the abdominal cavity, 4. the practice of removal of diaphragm and peritoneum, 5. the evisceration on the ground or in hanging position, 6. the order in which an animal was killed during the hunting day; and 7. the location where *Salmonella* spp. was found - skin, carcass meat and/or feces.

RESULTS

Prevalence of Salmonella spp. in wild boars

Table 2 presents overall data, including the epidemiological part of the study as well as *Salmonella* findings in feces (Petrović et al., 2022).

Table 2: Prevalence of *Salmonella* in each hunting estate (HE): prevalence in animals and positive samples

Hunting estates	No of animals in HE per year	Examined animals (% of population)	*Salmonella* positive animals (prevalence %)	*Salmonella* isolates (% of examined animals)			
				skin	carcass meat	lymph node	feces[2]
A	180	63 (35.0)	2 (3.2)	0	0	0	2 (3.2)
B	160	26 (16.3)	0	0	0	0	0
C	210	59 (28.1)	1 (1.7)	0	0	0	1 (1.7)
D	82	12 (14.6)	4 (33.3)	0	0	0	4 (33.3)
E	340	66 (19.4)	2 (3.0)	1 (1.5)	0	0	1 (1.5)
F	210	57 (27.1)	1 (1.8)	0	0	0	1 (1.8)
G	150	48 (32.0)	1 (2.1)	0	0	0	1 (2.1)
H	220	26 (11.8)	0	0	0	0	0
I	55	32 (58.2)	2 (6.3)	0	2 (6.3)	0	1 (3.1)
J	20	10 (50.0)	0	0	0	0	0
K[1]	35	15 (42.9)	5 (33.3)	2 (13.3)	2 (13.3)	1 (6.7)	2 (13.3)
L[1]	15	11 (73.3)	0	0	0	0	0
TOTAL	1677	425 (25.3)	18 (4.2)	3 (0.7)	4 (0.9)	1 (0.2)	13 (3.1)

[1] "K" and "L" are open hunting estates, all other estates have fence
[2] The results for feces were already published (Petrović et al, 2022)

The presence of *Salmonella* spp. was confirmed in 18 wild boars, with an overall prevalence of 4.2%. The highest prevalence was found in two estates, open estate "K" (33.3%) and fenced estate "D" (33.3%). Three animals had more than one *Salmonella* serotype detected. Serotyping of the 21 *Salmonella* isolates resulted in fifteen *Salmonella* Enteritidis isolates (71.4% of total number of isolates), five isolates of *Salmonella* Typhimurium (23.8%) and only one *Salmonella* Infantis isolate (4.8%).

Salmonella Typhimurium strain was detected both in feces and on the carcass meat of the same animal (hunting estate "I), so the total number of positive animals in this estate is two. In the hunting estate "K" the *Salmonella* Enteritidis strain was isolated from the skin and carcass, but not from the feces of the same animal. In all other examined animals, *Salmonella* was isolated from only one of the sampling sites. Prevalence of *Salmonella enterica* serotypes as percent of all examined samples was as follows: on carcass Enteritidis 0.7%, Typhimurium 0.2%; on skin Enteritidis 0.4%, Typhimurium 0.2 % and in feces Enteritidis 1.6%, Typhimurium 0.7%, Infantis 0.2%.

Influence of different factors on Salmonella findings

Several factors that influenced *Salmonella* presence were investigated. Rainfall during hunting increased findings of *Salmonella* on the skin ($\chi 2 = 9.18$, $p = 0.01$), but not on the carcass meat ($\chi 2 = 1.38$, $p = 0.5$). The possibility of detecting *Salmonella* on the skin significantly increased if boars had two or more entrance wounds ($\chi 2 = 6.30$, $p = 0.04$). The $\chi 2$ test indicated that the damage of the abdominal cavity caused by non-expert shooting, significantly increases the likelihood of finding *Salmonella* on the skin ($\chi 2 = 7.03$, $p = 0.03$), but it was not correlated with the findings on carcasses ($\chi 2 = 0.76$, $P = 0.73$). The practice of diaphragm and peritoneum removal did not significantly affect the finding of *Salmonella*. Furthermore, there was no significant difference between the evisceration being done on the ground/floor or in hanging position ($\chi 2 < 3.84$, $p > 0.05$), *Salmonella* was more commonly detected in feces (3.1%), than on the carcass meat (0.9%), skin (0.7%) or mesenteric lymph node (0.2%), but this difference is not statistically significant ($\chi 2 = 1.22$, $p = 0.54$).

Characterization of isolated Salmonella strains

The dendrogram carried out based on the Ward's linkage correlation coefficient obtained between the *S*peI and *Xba*I macrorestriction PFGE profiles (Figures 1 and 2), demonstrates the existence of 8 groups of isolates. The fol-

lowing groups of identical isolates according to both PFGE profiles were found (isolate numbers and origin are shown in table 1): group one (320, 321), group two (344, 346), group three (246, 248, 249), and big group four (254, 317, 319, 333, 336, 354, 355, 358, 376). Identical profiles of isolates 320 and 321 as well as isolate 319, which is identical to the whole group of isolates from feces (group four) were significant in proving the hypothesis that there was a cross contamination between feces and carcass/skin.
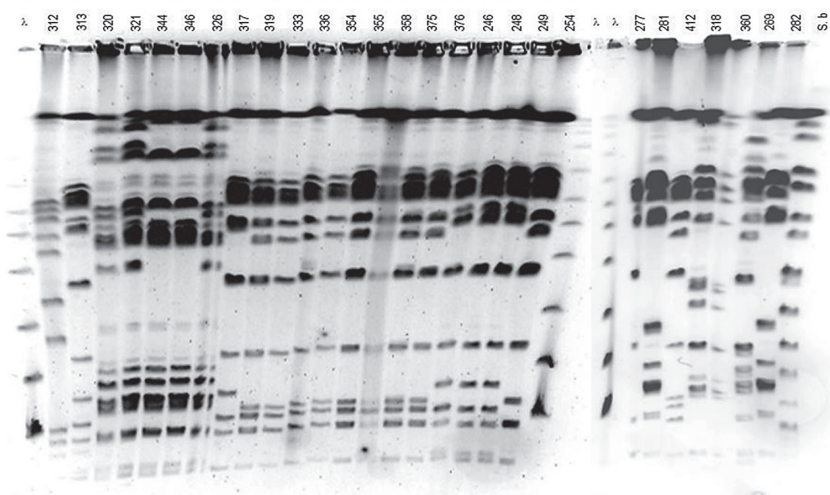

Figure 1: Pulsed-field gel electrophoresis (PFGE) macro-restriction fragment patterns of *Salmonella* sp. digested with *Xba*I enzyme.
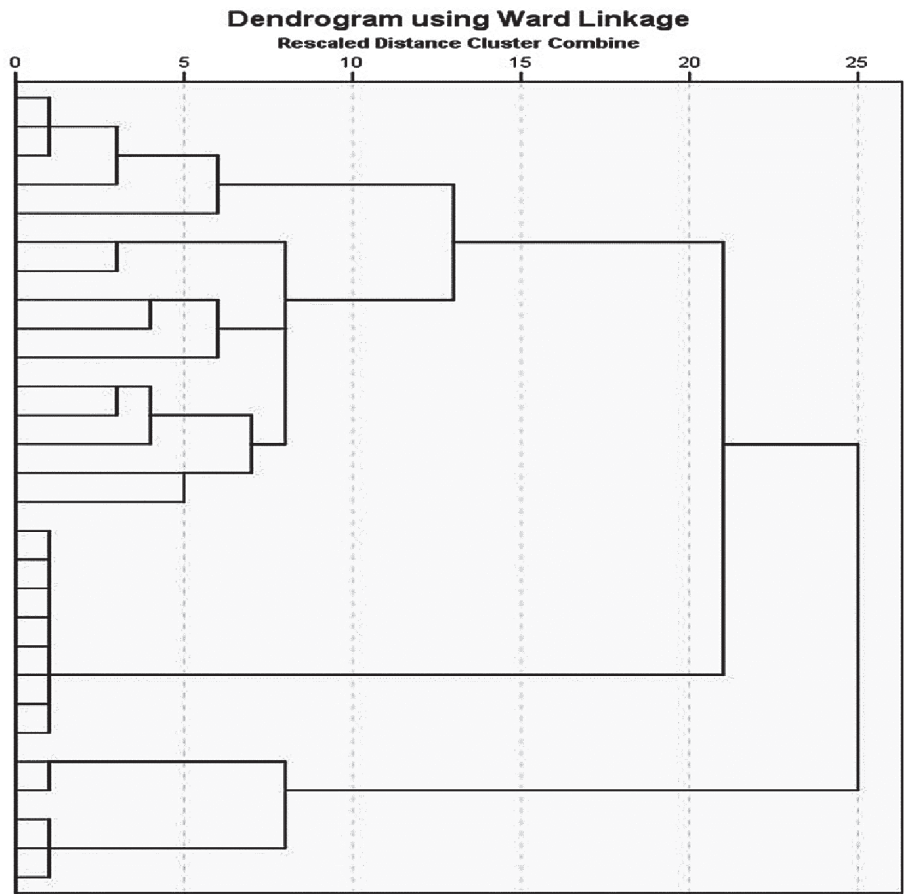
Figure 2: Dendrogram derived from the Ward linkage coefficient of correlation between the obtained PFGE *Xba*l macro-restriction profiles; Hunting estates are in quotation marks

DISCUSSION

A quarter of the wild boar population present at the tested location (25.3%) was examined for *Salmonella,* with an overall prevalence of 4.2%. The prevalence in wild boars is a little bit higher than the prevalence (2.0%) in farmed pigs from the same Vojvodina region (Stojanac et al., 2013). However, it is much lower than in finishing pigs from Hungary (up to 21.5%, Biksi et al., 2007) or free-range pigs from Spain (32.6%, Garrido et al, 2021). A relatively low prevalence (below 4%) was also found in other published studies. In one study in Italy, Orsoni et al. (2020), did not find *Salmonella* presence on car-

casses of hunted wild boars. On the contrary, one earlier study by Rodas et al. (2014) determined its presence in 3.6% of investigated meat samples. *Salmonella* was more commonly detected in animals from open hunting estates compared to fenced estates, because the presence of wild boars from open hunting estates in Vojvodina was noticed around waste where a large amount of improperly removed carcasses of domestic animals were dumped (estate "K") (Petrović et al. 2022).

The dominant serotype in this study was *Salmonella* Enteritidis (71.4%), while other serotypes are less prevalent. *Salmonella enterica* serotypes Enteritidis, Typhimurium and Infantis were also found in Switzerland, Portugal and Italy (Magnino et al., 2011; Vieira-Pinto et al., 2011; Wacheck et al., 2010), but other serotypes, like *S*. Diarizonae, *S*. Manhattan have been detected in some other studies (Rodas et al., 2014). Our present findings from wild boars prove that similar *Salmonella* serotypes are present in wildlife and in domestic pigs and poultry in the same geographical area, with *S*. Enteritidis, *S*. Infantis and *S*. Typhimurium frequently found in domestic animals (47.7%, 27.9% and 13.9%, respectively) (Petrović et al., 2015). These serotypes are also the most common isolates from human infections in Serbia according to the national data (Institute of Public Health of Serbia, 2014).

As for the sample site, feces yielded most of the isolated *Salmonella* (3.1%), and skin and carcass meat were significantly less contaminated (0.7% and 0.9%, respectively). *Salmonella* presence in feces is expected and indicates possible shedding of the pathogen, which can contaminate animal skin and subsequently carcass meat surface during handling and dressing procedures (Antić et al., 2011).

In all the tested animals, a *post mortem* inspection was performed and no signs of post-mortal lesions in *Salmonella* positive animals were found. *Salmonella* in wild boars is rarely manifested through clinical signs of the disease, yet an outbreak of wild boar salmonellosis with septicemia, caused by *Salmonella* Choleraesuis has been reported in Italy (Conedera et al., 2014).

The microbiological conditions of meat from hunted animals can be compromised by poor placement of shots (in the abdomen), the evisceration and dressing in the field without access to clean water, and ageing of carcasses at ambient temperatures (Gill, 2007; Paulsen, 2011; Mirčeta et al., 2017).

This study found that damage of the abdominal cavity caused by improperly placed shot significantly increases the possibility of finding *Salmonella* on the skin, but surprisingly not on the carcass meat. These findings are to some extent consistent with Atanassova et al. (2008) and Avagnina et al. (2012), who found that animals shot in any location posterior to the diaphragm can be

reasonably considered to be at higher risk of microbiological contamination of carcass meat (with general microbial load, but undoubtedly with pathogens as well) than those shot elsewhere (heart, head and neck, spine). However, these studies did not look into wild boar skin as an important source of carcass microbial contamination. Another interesting result from our study was that if an animal had been shot two or more times, there was a statistically significant increase in *Salmonella* findings on the skin but not on the carcass.

Rain during hunting increases the likelihood of finding *Salmonella* on the skin of the shot animals, probably due to a better transferability of bacteria to the swabs from the wet than from the dry skin, as demonstrated on cattle hides (Blagojević et al., 2012). On the other hand, rain was not found to increase *Salmonella* presence on carcasses (with the skin left on) immediately after evisceration - one of the reasons might be a different sampling method for skin and carcass meat (2.5 times smaller surface of carcass meat was sampled).

Interestingly, the removal of diaphragm and peritoneum, as a hygiene measure usually performed during the dressing of carcasses, had no statistical significance on the prevalence of *Salmonella* on the carcass meat. In addition, there was no difference between evisceration on the floor or while hanging. It has to be pointed out, however, that our investigation of risk factors for *Salmonella* presence is based on a very small number of contaminated skins and carcasses (0.7% and 0.9%, respectively), which pose a major limitation to the interpretation of the results.

The PFGE characterization of the *S.* Typhimurium isolates found in feces (isolate 320) and carcass meat surface of the same animal (isolate 321) was identical to indistinguishable PFGE profiles, according to S*pel* and X*bal*. This finding confirms that carcass meat contamination originated from the feces of the same animal. It was also observed that this animal had been shot expertly (i.e. one shot in the head) but eviscerated in a lying position in the field and without maintaining good hygiene practice (evisceration). Furthermore, *Salmonella* Typhimurium isolates 344 and 346 from the feces of the same animal were compared and no difference was found in the PFGE profiles. The same was the case between *Salmonella* Enteritidis isolates 246 and 248 from the same carcass. The comparison of the isolates from the same animal was done because animals may be infected with different *Salmonella* genotypes due to the various accesses to the food sources and contaminated environment (Piras et al. 2021).

Low *Salmonella* detection rate on wild boar carcass meat compared to the findings in feces as a primary source of carcass microbial contamination is to some extent expected. Despite the frequently poor carcass dressing practices

observed during our study, contamination of carcass meat with enteric pathogens may be often infrequent (Gill, 2007).

CONCLUSION

This study provides valuable data on the presence of *Salmonella* spp. in wild boar population from the flat regions of Vojvodina and also clarifies some gaps in knowledge related to the epidemiology of this important foodborne pathogen. The overall *Salmonella* prevalence in wild boars from hunting estates in Vojvodina region was 4.2%, with a dominating serotype being *S*. Enteritidis. This study also confirmed that wild boar carcass meat surface contamination originates from the feces of the same animal. Although the prevalence of *Salmonella* was not found to be high in wild boars, there is still likelihood of exposure of meat consumers to this pathogen. Significant factors that influenced *Salmonella* presence on wild boar skin and carcass meat were rainfall during the hunt, two or more shots, and non-expert shooting. Therefore, good hunting practices and education of hunters in hygiene practices are essential in reducing the risk of *Salmonella* exposure to consumers of wild boar meat.

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Authors' contributions

J.P. carried out literature research, designed a study, did the microbiology, analyzed the results and drafted the manuscript. J.M. carried out the sampling, data collection and analysis and statistics. M.V. and I.S. were involved in microbiology., R.A. was involved in data analysis. All authors read and approved the final manuscript.

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Conflict of interest

All authors declare that there are no financial and personal relationships with other people or organizations that could inappropriately influence our work.

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