Microbial evaluation of wild boar carcasses coming from control culling in the subalpine Prealpi Orobie area, northern Italy

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Abstract

This study tested 32 carcasses of control-culled wild boars in a subalpine area of Northern Italy between May and November 2021, delivered to three approved game meat establishments. Carcasses and organs were submitted to the detection of *Salmonella* spp., *Campylobacter* spp., *Listeria* spp., and *Listeria monocytogenes*; carcass surfaces were also sampled for microbial counts [total viable count (TVC), *Enterobacteriaceae*, *Escherichia coli*, coagulase-positive *Staphylococci*]. *Campylobacter* spp. was detected with high prevalence (90.9%) in the caecum as well as *Listeria* spp. and *L. monocytogenes* (37.5% and 25%, respectively), whereas only one animal harbored *Salmonella* spp. (3.8%). A low contamination rate was detected on the carcasses for *Campylobacter* spp. and *Listeria* spp. High mean TVC, *Enterobacteriaceae,* and *E. coli* counts were detected on the carcasses (5.90, 4.83 and 2.54 Log CFU/cm^2, respectively). Animal sex and weight exerted a weak effect on bacterial counts; the same was observed for the culling/sampling interval. Moderately higher counts were detected in animals culled with high (>15°C) environmental temperatures. Animals shot in the abdomen showed higher counts for all the parameters except for S*taphylococci*, and an increasing count of enteric bacteria was observed when considering head/neck, shoulder, chest, and abdomen locations , respectively. A significant difference among the plants was observed, independently from the other factors, thus stressing the importance of the application of hygiene procedures in approved game meat establishments to limit carcass contamination.

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Introduction

The remarkable increase in the wild boar population in Italy (ISPRA, 2023) represents both a potential safety problem and an opportunity for the consolidation of a meat production chain. Wild boars are usually hunted during a limited season but culling by gamekeepers ("selection" or "control culling") is performed throughout the whole year to limit their number. The carcasses obtained by control culling are mandatorily sent to an approved game meat establishment (GME), to complete slaughtering and carcass dressing, and to perform *post-mortem* inspection (Ministry of Health, 2021). The meat obtained can be marketed in the European Union market.

Wild boar meat can harbor foodborne pathogens, such as *Campylobacter* spp., *Salmonella* spp., and *Listeria monocytogenes,* as well as spoilage bacteria (Altissimi *et al*., 2023). The presence of animal carriers cannot be completely prevented; nonetheless, contamination of carcasses by environmental and fecal microorganisms can be limited during slaughtering. Differently from domestic animals, no microbiological criteria are set by Regulation 2073/2005 for wild game carcasses (European Commission, 2005).

The present study aimed to evaluate the hygiene of wild boar carcasses coming from control culling in a subalpine area of northern Italy; the effect of different factors on microbial contamination was also considered.

Materials and Methods

Experimental plan

A total of 32 wild boar carcasses (17 females and 15 males) were included in the study. The animals were culled during the period May-November 2021 in the *Prealpi Orobie* area (Lombardy, northern Italy) by professional gamekeepers; nine animals were live-trapped and shot, whereas the others were shot in the field. The animals were immediately bled and, in most cases, eviscerated; the carcasses and the viscera were then transported to three GMEs located near the culling area, indicated as plant A (nine carcasses), B (eight carcasses), and C (14 carcasses); a single animal was slaughtered in a private setting. The short distance between the culling area and the plants allowed to deliver the carcasses in a short time (mean of 75 min., with a range of 10-105 min.). At the GMEs, the carcasses were immediately refrigerated. Slaughtering, *post-mortem* inspection, and dressing were performed within the same day or, in case of night delivery, during the following day. The slaughtering technique applied was similar to that used for cattle: owing to the difficulty of removing hair from wild boars' skin and to the presumptive high contamination level of the hair, the carcasses were submitted to skinning. Each carcass was individually identified by plastic bands and hung in cold rooms at a set temperature of +2°C (with the same setting for the three plants) until sampling. The viscera were kept in a plastic bag with the same identification as the carcasses and maintained at the same temperature until sampling.

Detection and count of pathogens

For the detection of bacterial pathogens, the following organs/materials were aseptically taken at GME when slaughtering was completed: mesenteric lymph nodes and caecum content were withdrawn from 26 carcasses (9 at plant A, 7 at plant B, 9 at plant C, and the one taken in the private setting) for the detection of *Salmonella* spp. Caecum content taken from 22 carcasses (9 at plant A, 7 at plant B, 5 at plant C, and the one taken in the private setting) was also submitted to *Campylobacter* spp. detection and count; tonsils were taken from all the carcasses and submitted to the detection of *L. monocytogenes* and other *Listeria* spp. Sampling of caecum content was made by decontaminating the external surface with ethanol (70% v/v) and cutting the gut wall by using a sterile knife; the content was then put in a sterile 50 mL container; the other samples were taken by sterile knife and put in a sterile plastic bag. All the samples were refrigerated and immediately transported to the laboratory, where they were analyzed within 24 hours. The mesenteric lymph nodes, the tonsils, and the liver samples were submitted to external decontamination by ethanol (70% v/v); then, lymph nodes and tonsils were cut into small pieces by sterile scissors and put in a sterile stomacher bag.

For *Salmonella* spp. detection, the ISO 6579:2017 method was followed (ISO, 2017a); for the detection of *Campylobacter* spp., the ISO 10272-1:2017 method without enrichment was followed (ISO, 2017b), as suggested for samples with high natural *Campylobacter* counts; this method allows the simultaneous detection and count of the pathogen. For the detection of *Listeria monocytogenes*, the AFNOR BRD 07/4-09/98 method was applied (AFNOR, 1998); for the detection of *Listeria* spp., 100 µL of the enrichment broth [Fraser broth (Scharlab Italia, Lodi, Italy)] were inoculated on Palcam agar plates (Scharlab) and incubated at 37°C for 24-48 hours. Typical colonies were submitted to species identification by Listeria-ID Microgen™ (Gold Standard Diagnostic, Budapest, Hungary).

Evaluation of carcass contamination

Each carcass was aseptically sampled according to ISO 17604-2015 (ISO, 2015): rump, flank, brisket, and neck were sampled, as suggested for cattle carcasses. Indeed, the slaughtering technique used for wild boars was similar to that applied for cattle, including a dehiding phase. For microbial counts, destructive sampling was applied by sterile knife on a total surface of 20 cm^2 (a strip of 1×5 cm for each location); the four samples were pooled in a stomacher bag, diluted with sterile saline (NaCl 0.85% - tryptone 0.1%) and analyzed. The samples were submitted to the count of total viable bacteria (TVC) (ISO, 2003), *Enterobacteriaceae* (ENT) (ISO, 2017c), β-glucuronidase producing *Escherichia coli* (EC) (ISO, 2001), coagulase-positive *Staphylococci* (CPS) (ISO, 2021), *Listeria* spp. (Palcam agar, incubated at 37°C for 48 hours), and *Campylobacter* spp. (ISO, 2017b). The limit of detection of the count method was 1.0 Log UFC/cm^2 (*Listeria* and *Campylobacter*) or 2.0 Log CFU/cm^2 (other parameters). For *Salmonella* spp. detection, sampling was performed on a total surface of 400 cm^2 by a sterile sponge that was processed following the ISO 6579:2017 method (ISO, 2017a).

Data analysis

The data obtained were analyzed considering the influence of various factors, such as animal sex (Male/Female) and live weight (<10 kg, 10-30 kg, 30-50 kg, >50 kg), environmental temperature at the time of culling (<10°C, 10-15°C, >15°C), shooting/sampling interval (<12 h, 12-24 h, >24 h), shot location (head/neck, shoulder, chest, abdomen), and GME (A, B, C and private setting). The data were submitted to the exact Fisher test (detection prevalence) or to one-way analysis of variance (microbial counts); a significance threshold of p=0.05 was considered.

Results and Discussion

Animal population

The wild boar population was characterized by a wide variability of live weight (5-88 kg), and a limited difference between sexes (mean weight of 28.3 and 30.7 kg in females and males, respectively). The time between culling and sampling was very variable, ranging between 6 h 30' and 34 h (mean of 18 h 30'). Considering the shot location, nine animals were shot in the head/neck (all captured by live trapping), while seven, ten, and six animals were shot in the shoulder, chest, and abdomen, respectively.

Detection and count of pathogens

The detection rate of *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., and *L. monocytogenes* in the target organs and the prevalence of detectable counts of the same microorganisms on the carcasses are shown in Table 1. The prevalence of *Campylobacter* spp. in caecum content was very high (about 90%), as already reported by previous studies performed in Southern Europe, with values around or higher than 50% (Diaz-Sanchez *et al*., 2013; Kerkhof *et al*., 2022; Ziomek *et al*., 2023). A high *Campylobacter* count was also detected (mean: 6.47 log CFU/g, ranging from 2.30 to 7.51 Log CFU/g), suggesting the ability of the pathogen to grow efficiently in the boar gut environment. Our data showed higher values if compared to the study of Kerkhof *et al.* (2022). Previous data obtained from domestic pigs showed lower *Campylobacter* counts in the gut content after experimental infection (Rath *et al*., 2022). Nonetheless, a low prevalence of detectable *Campylobacter* counts (≥1 Log CFU/cm^2) on the carcasses was observed.

Salmonella spp. was isolated in just one animal (a male weighing 65 kg), both in caecum content and mesenteric lymph nodes. Our data indicate a lower prevalence of *Salmonella* spp. in gut content if compared to previous studies performed in Italy (7-25%), and a similar prevalence in mesenteric lymph nodes (Chiari *et al*., 2013; Stella *et al*., 2018; Cilia *et al*., 2021). The pathogen was never detected on the carcasses. This result was in agreement with previous observations, showing a very low contamination rate (0-2.5%) (Stella *et al*., 2018; Orsoni *et al*., 2019; Ranucci *et al*., 2021). *Listeria* spp. was detected in 37.5% of tonsil samples; eight of the samples harbored *L. monocytogenes*, while the other species identified were *L. ivanovii*, *L. seeligeri,* and *L. innocua*. A high prevalence of these microorganisms was already detected in the Mediterranean area (52-68% for *Listeria* spp., and 35-41% for *L. monocytogenes*), showing a similar species pattern (Stella *et al.*, 2018; Palacios-Gorba *et al*., 2021). These data could be justified by the wide environmental diffusion of *Listeria* spp., which can easily contaminate the mouth and throat of boars during rooting, a typical feeding habit of wild boars. As for the other microorganisms, a low prevalence of detectable counts (≥1 Log CFU/cm^2) on carcass surfaces was detected, confirming the possibility of preventing contamination from the target sites to the carcasses during culling and slaughtering.

Factors influencing the prevalence of pathogens

Due to the low number of positive samples, only data regarding *Campylobacter* spp. in the caecum content and *Listeria* spp./*L. monocytogenes* in the tonsils were analyzed. *Campylobacter* spp. was isolated with similar frequencies and counts in males and females (90% and 91.7%, mean counts 5.12 and 5.43 Log CFU/g, respectively). The weight of the animals did not show a significant effect, but higher counts were detected in the extreme weight classes (5.69-6.23 Log CFU/g in animals weighing >50 kg or <10 kg, *vs.* 4.59-5.11 Log CFU/g in the other classes). A significantly higher count (p=0.016) was detected in animals culled with higher environmental temperatures (5.97 *vs.* 4.28 Log CFU/g with temperatures >15°C and <15°C, respectively). Our data partially agree with previous findings: some studies detected a higher prevalence in males, whereas the influence of animal weight is more debated; contrasting effects on *Campylobacter* counts by the environmental temperature are described (Diaz-Sanchez *et al.*, 2013; Carbonero *et al*., 2014; Ziomek *et al*., 2023). Some differences between animal sexes, also if not significant, were detected for *Listeria* spp. and *L. monocytogenes* (40% *vs.* 35.3%, and 33.3% *vs.* 17.6% in males and females, respectively), but a higher detection rate of *Listeria* spp. was observed in animals weighing more than 50 kg (83.3% *vs.* 33.3% in the other weight classes). A higher prevalence was detected at environmental temperatures >15°C (76.9% *vs.* 28.6% at lower temperatures); the influence of these factors is not clear and was not confirmed by previous data obtained in a similar area (Stella *et al*., 2018).

Evaluation of carcass contamination

The counts obtained from the carcasses are reported in Table 2. The mean TVC was higher if compared with those obtained from previous studies performed in Italy (5.90 *vs.* 3.2-4.6 Log CFU/cm^2). A similar result was obtained for *Enterobacteriaceae* (4.83 *vs*. 1.3-3.0 Log CFU/cm^2) and *E. coli* (3.92 *vs.*1.3 Log CFU/cm^2) (Avagnina *et al*., 2012; Stella *et al*., 2018; Cenci-Goga *et al*., 2021; Ranucci *et al*., 2021), whereas low counts were observed for CPS. In order to evaluate the results obtained, a comparison with the threshold set by Regulation No. 2073/2005 for process hygiene criteria was made. As already done by previous authors (Orsoni *et al*., 2020, Ranucci *et al*., 2021), threshold values set for pig carcasses were used (M=5.0 and m=4.0 Log CFU/cm^2 for TVC and M=3.0 and m=2.0 Log CFU/cm^2 for ENT): the application of such thresholds (higher than those applied to other ungulates) is based on the hypothesis that the carcasses coming from hunted animals leaving in non-controlled condition can result in higher counts, also if a proper dressing technique (including skinning) is applied. Following this approach, no "satisfactory" results were obtained for TVC, with five carcasses falling within the "acceptable" category and the other 27 classified as "unsatisfactory"; a similar pattern was observed for ENT (one "satisfactory", two "acceptable" and 29 "unsatisfactory" results). These data suggest the need for an improvement in hygiene procedures throughout the whole culling-slaughtering process.

Factors influencing carcass contamination

The effect of animal sex and weight, environmental temperature, culling-sampling interval, shot location, and GME on the microbial counts was analyzed (Table 2).

Animal sex and weight did not show an evident influence on bacterial counts, also if slightly higher values were observed in heavier animals (>50 kg). Previous studies gave variable results: higher counts were in some cases detected in heavier animals, due to the difficulty of carcass skinning, but on the opposite, lighter carcasses are more easily contaminated by the shot or during evisceration (Stella *et al*., 2018; Cenci-Goga *et al*., 2021; Ranucci *et al*., 2021).

The environmental temperature at the time of shooting had a slight effect on counts; anyway, higher values were observed at temperatures >15°C. This finding was in agreement with previous studies (Paulsen and Winkelmayer, 2004; Ranucci *et al*., 2021); nevertheless, the influence of temperature should be considered in combination with the hunting technique and the time needed to recover the animals.

The time interval between shot and carcass sampling did not

Table 1. Prevalence of the target microorganisms in the organs and on the carcasses.

	Organs - detection rate	Carcasses	
		Detection rate	Prevalence of counts ≥1.0 Log CFU/cm^2)
Campylobacter spp.	20/22 (caecum)	-	2/32
Salmonella spp.	1/26 (caecum) 1/26 (mes. lymph nodes)	0/32	-
Listeria spp.	12/32 (tonsils)	-	1/32
Listeria monocytogenes	8/32 (tonsils)	-	0/32

clearly influence the contamination level: it must be noted that this interval includes several phases that are known to influence the microbial counts (shot-bleeding-evisceration-refrigeration-skinning intervals), but limited information about the single intervals could be achieved.

As shown in Table 2, animals shot in the abdomen showed higher TVC, ENT, and EC counts. A shot in the abdomen could result in damage to the gastrointestinal tract, with spilling of stomach/gut content; moreover, such a shot could result in the delayed death of the animal. Considering separately the four shot locations, an increasing trend of the counts of ENT and EC was observed when proceeding forward on the animal body (Figure 1); such a trend was previously observed by Ranucci *et al.* (2021), but the importance of shot location is still debated (Avagnina *et al*., 2012; Mirceta *et al*., 2015; Cenci-Goga *et al*., 2021).

Finally, the influence of the GME receiving the carcasses was evaluated. A significant difference among the plants was observed, with GME C showing the highest values, in particular for ENT and EC ($p<0.01$). To show the influence of the plant procedures, the data were analyzed considering separately the animals shot in the abdomen (22.2%, 0%, and 28.6% in GME A, B, and C, respectively) and the others. In carcasses shot in the abdomen, higher counts were detected in plant C *vs.* A for all the parameters (differences of 0.70, 1.35, 1.05, and 0.65 Log CFU/cm^2 for TVC, ENT, EC, and CPS, respectively); on the other carcasses, higher counts in plant C were confirmed for ENT, EC, and CPS, whereas higher TVC values were detected in plant B. These results suggest a difference in the efficiency of the prevention of carcass contamination among the GMEs considered: in this light, it has to be noted that all the GMEs were equipped with modern slaughtering devices (mobile platform) and could efficiently refrigerate the carcasses; moreover, in all the plants not only wild boars were slaughtered (cattle and wild ungulates were slaughtered, too). Thus, the observed difference was supposed to be linked to the management of the carcasses and the hygiene measures applied; at plant C, the carcasses were often dressed at the end of the working day, thus being exposed to environmental contamination and high temperature, whereas this was occasionally observed in plant A and was never observed in plant B. This approach was applied as wild boar carcasses are considered "dirty" matter and are preferably dressed after the slaughtering of other animals (*e.g.*, cattle). The dressing operation was not observed during the sampling; thus, the personnel hygiene procedures could not be evaluated; in this light, it has to be noted that all the staff involved in carcass dressing had undergone the required training concerning hygienic practices to be applied during slaughtering.

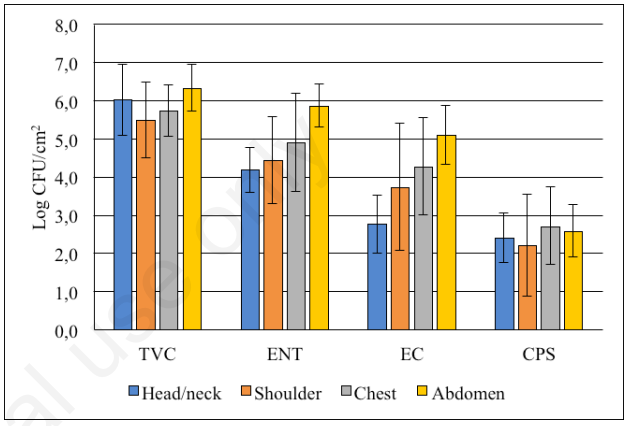

Figure 1. Effect of shot location on the counts obtained from the wild boar carcasses. TVC, total viable count; ENT, *Enterobacteriaceae*; EC, *Escherichia coli*; CPS, coagulase-positive *Staphylococci.*

Table 2. Microbial counts obtained from wild boar carcasses at the end of slaughtering procedures (Log CFU/cm2) and the effect of the influencing factors considered.

		TVC	ENT	EC	CPS
Sex	Female	5.95±0.92	4.91±1.53	3.98±1.41	2.60±0.76
	Male	5.83±0.59	4.75±1.28	3.84±1.31	2.48±0.61
Weight	<10 kg	5.36^{b}±0.66	4.45±1.19	3.99±1.31	2.25±0.55
	10-30 kg	6.22^{a}±0.77	5.01±1.39	3.86±1.38	2.57±0.81
	30-50 kg	5.58±0.66	4.53±1.54	3.49±1.45	2.63±0.59
	>50 kg	6.20±0.70	5.27±1.61	4.53±1.27	2.65±0.72
Shot location	Abdomen	6.32±0.64	5.86^{a}±1.32	5.10^{a}±1.01	2.58±0.68
	Other locations	5.80±0.78	4.60^{b}±1.33	3.64^{b}±1.27	2.53±0.70
Environmental temperature	<15°C	5.57±0.71	4.31±0.96	3.44±1.14	2.07^{b}±0.26
	>15	6.02±0.77	5.04±1.51	4.10±1.39	2.72^{a}±0.71
Culling-sampling interval	<12 h	6.24^{a}±0.79	4.55±1.21	3.20^{B}±1.21	2.21b±0.47
	12-24 h	5.94^{a}±0.67	5.39^{a}±1.30	4.56^{A}±1.18	2.78a±0.75
	>24 h	5.20^{b}±0.65	3.81^{b}±1.43	3.39±1.31	2.44±0.60
Plant	GME - A	5.37^{Bb}±0.60	4.12^{B}±1.25	3.83±1.26	2.28^{b}±0.54
	GME - B	6.17^{a}±0.85	4.29^{B}±0.98	2.75^{B}±0.74	2.09^{B}±0.18
	GME - C	6.18^{A}±0.58	5.75^{A}±1.20	4.77^{A}±1.07	3.01^{Aa}±0.69
	Private setting	4.48	2.78	2.00	<2.00
Total		5.90±0.77	4.83±1.40	3.92±1.35	2.54±0.68

^{A,B}Significant difference among the categories (A>B, $p<0.01$); ^{a,b}significant difference among the categories (a>b, $p<0.05$); TVC, total viable count; ENT, *Enterobacteriaceae*; EC, *Escherichia coli*; CPS, coagulase-positive *Staphylococci*; GME, game meat establishment.

The importance of the application of proper slaughtering procedures by trained operators has been stated by other authors (Mirceta *et al*., 2015; Orsoni *et al*., 2020). This was further confirmed, in our study, by the detection of higher counts in plant B *vs.* A, although plant A received quite all carcasses from live-trapped animals shot in the head. The single carcass processed in a private setting showed the lowest contamination level; also, if this result could not be representative, it suggests the possibility of performing a hygienic slaughtering also in simple, not approved, facilities, as observed in a previous study (Stella *et al*., 2018).

Conclusions

The increase in the wild boar population in northern Italy poses attention to the safety and hygiene of the growing amount of meat available on the market. Previous data highlighted the importance of the adoption of proper hygiene procedures during culling and slaughtering/dressing: the mandatory delivery of the carcasses to approved GME represents an opportunity to warrant the control by the competent authority and the use of appropriate equipment, but only the adoption of hygiene procedures allows to obtain a carcass contamination level quite comparable with that of domestic animals.

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