Research Note

Microbiological Evaluation of Carcasses of Wild Boar Hunted in a Hill Area of Northern Italy

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ABSTRACT

This study evaluated the prevalence of potential pathogenic bacteria (mainly *Campylobacter* spp., but also *Listeria monocytogenes* and *Salmonella*) in wild boar (*Sus scrofa*) and the hygiene of carcasses of wild boar hunted in a hill area of northern Italy during a hunting season (October to December). In total, 62 animals were submitted to microbiological analyses of the tonsils (detection of *Listeria* spp. and *Listeria monocytogenes*), caecal content (detection of *Salmonella* and *Campylobacter* spp.), mesenteric lymph glands (detection of *Salmonella*), and carcasses. In addition to analyzing pathogen prevalence and carcass hygiene of these animals, we performed an enumeration of total viable count (TVC), *Enterobacteriaceae, Escherichia coli,* coagulase-positive staphylococci, and spores of sulfite-reducing clostridia. Influencing factors considered were sex, weight, and age of the animals and environmental temperature on the day of hunting. A high prevalence was observed for *L. monocytogenes* in tonsils (35.3%) and for *Campylobacter* spp. in caecal content (51.8%), whereas *Salmonella enterica* strains (mainly serovar Thompson) were only occasionally isolated (7% in caecal content and 3.5% in lymph glands). The prevalence of *L. monocytogenes* was influenced by animal age and environmental temperature. *Campylobacter* spp. were the only pathogens detected on the carcasses (16.7%). Carcasses were characterized by low levels of contamination: TVC, 3.21 ± 0.80 log CFU/cm^2, *Enterobacteriaceae,* 1.32 ± 0.89 log CFU/cm^2; *E. coli,* 1.31 ± 0.93 log CFU/cm^2; and occasional detection of low counts of staphylococci and clostridia. TVC was positively influenced only by high environmental temperature, and higher *Enterobacteriaceae* counts were detected on heavy male carcasses than on females. The results confirmed the potential role of wild boars as reservoirs for the most important foodborne pathogens. But a low carcass contamination level is achievable if hunters are properly trained about hygienic carcass management and slaughtering procedures.

Key words: *Campylobacter*; Carcass contamination; *Listeria*; *Salmonella*; Wild boars

In the past 50 years, a huge increase in the wild boar (*Sus scrofa*) population has occurred in several European countries *(22, 39, 42)*. In Italy, wild boar has been diffusing over the past several decades, and today it is the most widespread wild ungulate, with a presence in two-thirds of the National Territory *(7, 38)*. This diffusion is mainly because of its high prolificity, the favorable climatic conditions, and the depopulation of Apennine and Alpine areas, previously used for agriculture and animal rearing. Also, the massive introduction of boars from foreign countries or from farms has played a role in the increase in wild boar numbers *(5, 22, 38, 43)*. Consequently, an increase in hunted wild boars in Italy is observed, reaching annually above 150,000 animals *(41)*.

Wild boar hunting usually occurs during the fall-to-winter period or episodically in other periods for numerical reduction. The animals are usually hunted by driving toward the hunters (e.g., with dogs) or stand hunting, and these practices may influence the hygiene of the meat obtained *(19, 20)*. In the case of dog hunting, the injuries caused in the wild boar often do not affect vital organs, resulting in a potential diffusion of microorganisms in the whole carcass or in a rupture of contaminated viscera (e.g., the gut) *(35, 38)*. Stand hunting seems to cause higher possibilities of microbial spread compared with dog hunting.

The production of wild boar meat for self-consumption or for the supply of local retailers and restaurants is not submitted to the requirements stated by the European legislation *(17),* such as, for example, slaughtering room prerequisites or microbiological process hygiene criteria *(13)*.

The hygiene of hunted boar meat is often affected by factors such as the lack of sanitary controls in wild populations that can host many potential pathogenic bacteria and the application of improper slaughtering and transport procedures *(15)*.

Thus, the aim of this study was to evaluate the prevalence of the main potential pathogenic bacteria, *Campylobacter* spp., *Salmonella,* and *Listeria monocytogenes,* in wild boars hunted in Oltrepò Pavese, a typical hill area of Northern Italy. The microbial population of the

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TABLE 1. *Characteristics of the hunted boars*

	Minimum	Maximum	Mean	Median
Age (mo)	4	72	21.5	20
Wt (kg)	17	131	61.2	60
Wt frequency distribution (kg):	<30	30–60	60–90	>90
No.	10	22	21	9

carcasses was also evaluated to highlight the suitability of the artisanal slaughtering practices.

MATERIALS AND METHODS

Hunting conditions and animals. This study was performed in a hill area of about 2,700 ha in Oltrepò Pavese (northern Appennines, Lombardy), Italy. The wild boars were hunted by the "driving" technique by a single team within a regular hunting season (October to December) during 16 hunting sessions. In total, 62 animals were submitted to microbiological analyses. The population was composed of 31 males and 31 females. Animals were classified by sex, age, and weight. Age data were determined from examination of the teeth according to the scheme of Cavenago and Geremia (9). All classification data are reported in Table 1.

After killing the boars, they were left on the ground until the end of the hunt, thereby being exposed to environmental conditions from 1 to 3 h. Each animal was identified by the application of a metal clamp placed around the calcaneal tendon. Carcasses were then transported by a truck to the dedicated structure where slaughtering operations were performed by a single operator at the end of the day in a closed area. Carcasses were skinned, eviscerated, and divided in sides. Carcasses were then transported (10 to 15 min) to a refrigerated room (4°C) and hung for 3 to 4 days before being sectioned, frozen, and portioned for self-consumption.

Sampling and detection of target bacteria. During the evisceration, the following organs were obtained and inserted in sterile bags for subsequent analyses: tied caecum (detection of *Salmonella* and thermophilic *Campylobacter* spp.), mesenteric lymph glands (detection of *Salmonella*), and tonsils (detection of *L. monocytogenes* and other *Listeria* spp.). Samples were transported in refrigerated conditions to the laboratory and analyzed within 24 to 36 h after slaughtering.

For the detection of *Salmonella* in the caecal content, the external surface of the caecum was disinfected, and then the caecal content was isolated and analyses were performed following ISO 6579-2002 (26). The serogroup of the *Salmonella* isolates was determined by slide agglutination tests with O antigen and H antigen antiserums; results were interpreted in agreement with the Kauffmann-White scheme (23). For the detection of thermophilic *Campylobacter* spp., each sample was inserted into tubes containing 10 mL of Bolton broth (with 5% laked horse blood added; Thermo Fisher Diagnostics, Rodano, Italy), and analyses were performed according to ISO 10272-1 (31). A parallel method, as indicated by Steele and McDermott (44), with some modifications, was also applied. In brief, 300 μL of the enriched Bolton broth was put onto a 0.45-μm-pore-diameter cellulose ester membrane filter (Sigma-Aldrich Italy, Milan), previously placed onto the surface of a blood agar plate (tryptone soy agar plus 5% of defibrinated sheep blood; Thermo Fisher Diagnostics). After 45 min (time needed to allow *Campylobacter* spp. to pass through the membrane), the filter was removed and the remaining share was

distributed by sterile loops. The plates were incubated for 48 h at 42°C under microaerobic conditions (Anaerogen sachet, Thermo Fisher Diagnostics) in closed jars. The isolated colonies were confirmed as described in ISO 10272-1. Mesenteric lymph glands were surface disinfected, cut into small pieces, inserted in a stomacher bag with 100 mL of buffered peptone water, and homogenized by a stomacher (Interscience, Saint Nom, France) for 1 min. Detection of *Salmonella* was performed as previously described (26).

For the detection of *Listeria* spp. and *L. monocytogenes,* tonsils were cut and 5 to 10 g was inserted into a stomacher bag with Half-Fraser broth (1:10; Thermo Fisher Diagnostics). Detection of *L. monocytogenes* was performed according to the AFNOR BRD 07/04–09/98 method (2). For the detection of *Listeria* spp., Palcam agar plates (Biogenetics, Ponte San Nicolò, Italy) were inoculated in parallel with the enrichment broth and incubated at 37°C for 48 h.

Evaluation of carcass hygiene after slaughtering. At the end of slaughtering procedures, nondestructive samplings were performed by the double swab method (27) on four areas of 100 cm² each. The areas were chosen from the most representative areas for carcass contamination: rump, flank, brisket, and neck. The four swabs taken from each carcass were pooled, put into a unique sterile stomacher bag with diluent solution (0.85% NaCl, 0.1% peptone), and homogenized. Serial 10-fold dilutions were prepared and plated for the evaluation of the following parameters: total viable count (TVC) (28), *Enterobacteriaceae* (30), *Escherichia coli* (25), coagulase-positive staphylococci (CPS) (24), and spores of sulfite-reducing Clostridia (29), with previous treatment of the samples at 80°C for 10 min. The results for TVC and *Enterobacteriaceae* were compared with the thresholds ("process hygiene criteria") set by Commission Regulation (EC) No 2073/2005 (16) for bovine carcasses (as slaughtering procedures are similar). These limits were modified to adapt to the nondestructive method as required by Italian State-regions agreement 41/2016 (12): the m and M values intended for the distinction among "satisfactory" (mean log CFU/cm² < m), "acceptable" (m < mean log CFU/cm² < M), and "unsatisfactory" (mean log CFU/cm² > M) results were reduced to 20% of those indicated by the regulation.

For the detection of *Campylobacter* spp., *Listeria* spp., and *L. monocytogenes,* two areas (rump and neck) of 100 cm² each were sampled by sterile swabs inserted into tubes containing 10 mL of specific broth (Bolton broth for *Campylobacter* spp. and Half-Fraser broth for *Listeria* spp.; Biogenetics). Then, for the detection of *Campylobacter* spp., the ISO 10272-1 method was applied (31); the detection of *L. monocytogenes* was performed by the AFNOR BRD 07/04-09/98 method (2), in parallel with the detection of *Listeria* spp., as described in the previous section. For the detection of *Salmonella,* sterile sponges were swabbed on two 100-cm² areas (near the areas used for the other withdrawals) and then pooled and inserted in a stomacher bag with 100 mL of buffered peptone water. The further steps were performed following the ISO 6579-2002 method (26).

Statistical analysis. Data obtained from the detection of the target microorganisms were submitted to chi-square test or Fisher exact test (applying the Yates correction), whereas the microbial counts were analyzed by analysis of variance using SAS software (SAS Institute Inc., Cary, NC). The following factors were considered: sex, age, and weight of the animal and environmental temperature on the day of hunting. Moreover, the correlation

TABLE 2. *Prevalence of* Campylobacter *spp.,* Salmonella, Listeria *spp., and* L. monocytogenes *in the hunted boar organs and on the carcasses*

Microorganism	No./total no. (%) in organs and on carcasses			
	Tonsils	Caecal content	Lymph glands	Carcasses
Campylobacter spp.	—[a]	29/56 (51.8)	—	5/30 (16.7)
Salmonella enterica	—	4/57 (7.0)	2/57 (3.5)	0/30 (0)
Listeria spp.	37/54 (68.5)	—	—	8/30 (26.7)
L. monocytogenes	18/51 (35.3)	—	—	0/30 (0)

[a] —, not performed.

between the detection of a target microorganism in the organs and on the carcass of the same animal was evaluated.

RESULTS AND DISCUSSION

The interest of consumers in wild game meat has been increasing for the past several years *(4),* with wild boar meat representing the most consumed game meat in Italy (about 80% of the meat obtained from wild ungulates). Ramanzin et al. *(41)* estimated a supply of wild boar meat in Italy of more than 5,000 tons. Today, wild boar meat is not only consumed within hunter families; it is also consumed by consumers at local restaurants who value this product for its sensorial characteristics and its flavor that is reminiscent of traditional link to the territory.

Prevalence of target bacteria in wild boars. The results of the prevalence analyses performed are shown in Table 2. The data confirmed the high prevalence of wild boars acting as potential carriers of pathogenic bacteria, as already reported in previous studies *(6, 11, 32, 47, 49, 52).*

L. monocytogenes was isolated in a high number of animals (about one-third). Other *Listeria* spp. were detected with even higher frequency. Previous studies indicated a variable prevalence of *Listeria* spp. and *L. monocytogenes* in the tonsils and feces of wild boars *(32, 47, 49).* These data suggest the role of wild boars as biological sentinels via their feeding behavior.

Salmonella enterica was detected in six animals (10.5%) and was mainly isolated from caecal content; the presence of this pathogen in the lymph gland samples confirmed its transfer from the gut through the local lymphatic vessels, as already reported for domestic pigs *(18);* however, in our case, no connection between positive samples from caecal contents and lymph gland was observed. *Salmonella* prevalence observed in the present study is in agreement with that reported in another study performed in Italy *(52),* although Chiari et al. *(11)* reported higher values (almost reaching 25%) in animals collected from another area of the same region (Lombardy). Five of the six *Salmonella* isolates in our study (five from caecal content and one from lymph glands) were identified as serovar Thompson, and the other isolate was identified as *Salmonella* Braenderup. *Salmonella enterica* subsp. *enterica* serovar Thompson has been frequently isolated from wild boar *(11, 34, 52)* and widespread in several other animal species. Its role as a human pathogen has been recognized, as a severe outbreak linked to the consumption of

Salmonella Thompson–contaminated smoked salmon occurred in The Netherlands, with more than 900 people involved *(21). Salmonella* Braenderup is known as being responsible for foodborne disease and has been occasionally isolated from many animal species, including domestic and wild pigs *(10, 45, 46, 48).*

The presence of *Campylobacter* spp. was revealed in a high percentage of animals (>50%), confirming the results of previous studies *(6, 40).* To evaluate the effect of the potential influencing factors, the data were analyzed by taking into account the sex and weight of the animals and also the environmental temperature on the day of hunting.

The sex of the animals had no significant effect on the prevalence of the pathogens in the target organs (Table 3). *L. monocytogenes* was found in a higher percentage of females, whereas the other target organisms were isolated more frequently from males, but no statistically significant differences were observed for any of the pathogens. Wacheck et al. *(47)* observed a higher prevalence of the pathogens in females than in males, suggesting a role of the specific social behavior as females live mainly in groups and males often live alone (but these findings were not in agreement with those of other studies *(11, 14, 52)*).

The age of the animals did not have a significant impact on the prevalence of *Listeria* spp. and *L. monocytogenes,* although higher values were reported in the subadults than in adults. The prevalences of *Campylobacter* spp. and *Salmonella* were not influenced by the age classes, whereas in previous studies a significantly higher prevalence of *Salmonella* in young animals was reported *(11, 52).* Also, the weight of the animals did not have a significant influence on the microbial prevalence. A high *Listeria* spp. detection rate was found in heavier animals (>90 kg) than in lighter animals, probably because of the presence of some subadult animals in the highest weight class.

Finally, the environmental temperature on the day of hunting showed no significant trend in bacterial prevalences, although *Listeria* spp. and *L. monocytogenes* were progressively more frequent when the temperature decreased. This aspect, previously described for *Campylobacter* spp. by Carbonero et al. *(6),* needs to be further elucidated, as cold temperatures should be less permissive to bacterial replication, but other factors (e.g., higher moisture, different behavior, or animal density) could have a strong influence on *Listeria* diffusion.

TABLE 3. *Prevalence of the target bacteria in the organs and on the carcasses of the hunted boars as influenced by sex, age, and weight of the animals and environmental temperature[a]*

Parameter	Organ				Carcass	
	Campylobacter spp.	*Salmonella enterica*	*Listeria* spp.	*L. monocytogenes*	*Campylobacter* spp.	*Listeria* spp.
Sex						
Female	13/27 (48.1)	1/28 (3.6)	17/26 (65.4)	11/25 (44.0)	2/15 (13.3)	4/15 (26.7)
Male	16/29 (55.2)	5/29 (17.2)	20/28 (71.4)	7/26 (26.9)	3/15 (20.0)	4/15 (26.7)
Age (mo)						
<12	9/17 (52.9)	3/17 (17.6)	10/16 (62.5)	4/15 (26.7)	2/10 (20.0)	2/10 (20.0)
12–36	14/28 (50.0)	3/29 (10.3)	21/27 (77.8)	11/26 (42.3)	1/16 (6.2)	5/16 (31.2)
>36	6/11 (54.5)	0/11 (0)	6/11 (54.5)	3/10 (30.0)	2/4 (50.0)	1/4 (25)
Wt (kg)						
<30	5/9 (55.6)	1/9 (11.1)	5/9 (55.6)	1/8 (12.5)	2/5 (40.0)	1/5 (20.0)
30–60	10/20 (50.0)	2/20 (10.0)	13/19 (68.4)	8/19 (42.1)	0/10 (0)	3/10 (30.0)
60–90	9/19 (47.4)	2/20 (10.0)	13/18 (72.2)	8/17 (47.1)	2/10 (20.0)	3/10 (30.0)
>90	5/8 (62.5)	1/8 (12.5)	6/8 (75.0)	1/7 (14.3)	1/5 (20.0)	1/5 (20.0)
Temp (°C)						
<10	6/13 (46.2)	2/14 (14.3)	11/13 (84.6)	7/13 (53.8)	0/5 (0)	1/5 (20.0)
10–15	17/27 (63.0)	1/27 (3.8)	15/23 (65.2)	8/20 (40.0)	2/14 (14.3)	1/14 (7.1)*[b]
>15	6/16 (37.5)	3/16 (18.7)	11/18 (61.1)	3/18 (16.7)	3/11 (27.3)	6/11 (54.5)*

[a] Values presented as number/total number (%).
[b] * $P < 0.05$.

Prevalence of target bacteria on carcasses. As shown in Table 2, only *Campylobacter* spp. (16.7%) and *Listeria* spp. (26.7%) were detected on the carcasses; no *Salmonella* or *L. monocytogenes* was detected. The variable prevalence of pathogens on wild boar carcasses or meat has been observed previously. For example, the absence of *L. monocytogenes* on the carcasses in our study confirmed the findings of other studies (3, 32, 40). *Salmonella* has been generally isolated in low rates (0 to 7%) from wild boar carcasses (3, 11), whereas contamination of the carcasses by *Campylobacter* was described as more variable (2 to 24%) (3, 32, 51).

Carcass contamination during slaughtering, even if reduced by careful working practices, cannot be completely avoided. In the present study, cross-contamination among carcasses could be hypothesized because of a lack in equipment disinfection, as two of the five *Campylobacter* spp.–positive carcasses and three of the eight *Listeria* spp.–positive carcasses were obtained from animals with negative samples from the caecum or the tonsils. Theoretical contamination transfer rates (prevalence in carcasses or organs) were calculated for *Campylobacter* spp. (32%) and *Listeria* spp. (39%).

The analysis of the influencing factors (Table 3) did not reveal a significant impact of sex, age, or weight of wild boars on the microbial prevalences on their carcasses. Increased prevalences of *Campylobacter* spp. and *Listeria* spp. were instead detected when higher environmental temperatures were measured, in particular, a significantly higher ($P < 0.05$) prevalence of *Listeria* spp. was detected with environmental temperatures above 15°C.

Bacterial contamination of carcasses. The microbiological quality of game meat is strongly affected by hunting, transport, and slaughtering procedures (8, 36, 41). In particular, the contamination of the carcasses at the end of the slaughtering process is strictly related to the hygienic manufacturing procedures (mainly skinning and evisceration).

In this study, general, hide, and enteric contamination indicators were considered (Table 4). The mean TVC values obtained indicated a good hygiene level of the carcasses, with 90% of the samples characterized by bacterial loads within the range 1.0 to 4.2 log CFU/cm^2, without values higher than 5 log CFU/cm^2 (Fig. 1). These loads were comparable or lower than those reported in previous studies (3, 37, 40). Low loads of *Enterobacteriaceae* on the

TABLE 4. *Bacterial numbers on the surface of wild boar carcasses*

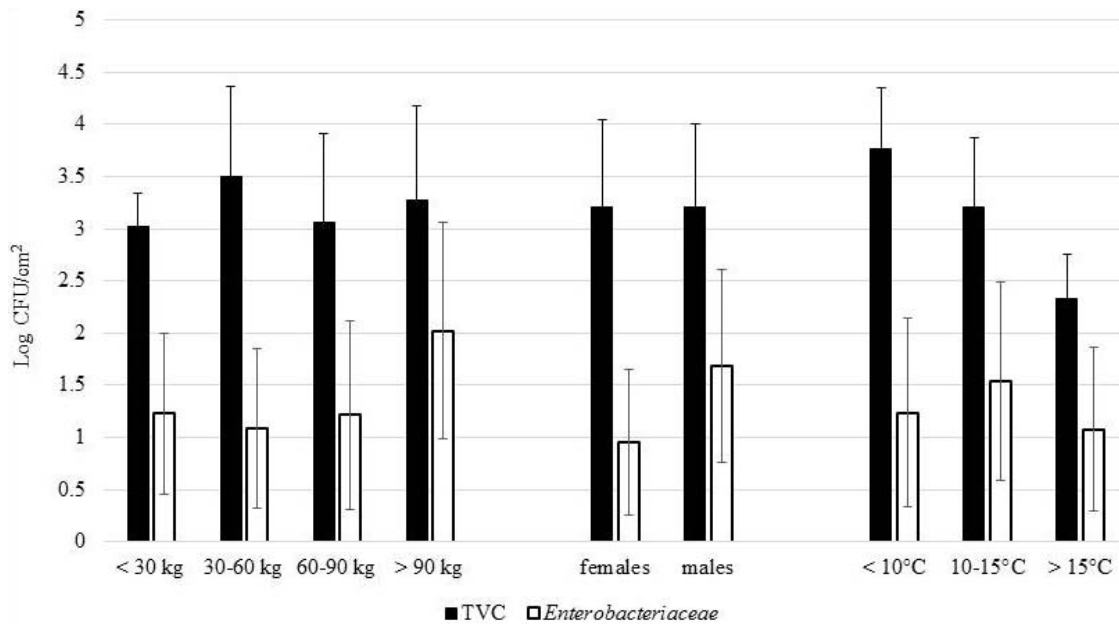|	TVC	*Enterobacteriaceae* (log CFU/cm^2)	*E. coli* (log CFU/cm^2)	CPS	Spores of sulphite-reducing clostridia
Mean	3.21	1.32	1.31	<1.00	<1.00
SD	0.80	0.89	0.93	—[a]	—
Median	3.37	1.27	1.26	<1.00	<1.00

[a] —, not applicable.

FIGURE 1. *Mean TVC and* Enterobacteriaceae *counts on the carcass surfaces of wild boars, as influenced by sex and weight of the animals and environmental temperature.*

carcasses indicated the proper application of hygienic practices without significant fecal contamination: 43% of the samples had counts below 1 log CFU/cm^2, whereas higher values were noted in other studies *(3, 33, 37, 40)*. As reported by Lagrange and Schmidt *(33),* almost the whole *Enterobacteriaceae* population was represented by *E. coli*. The contaminations by coagulase-positive staphylococci and sulfite-reducing clostridia were sporadic, being present in just 2 of 30 and 6 of 30 samples, respectively, with loads always below 1 log CFU/cm^2.

In our study, partial evisceration (removal of the gastrointestinal tract) was always performed within a short time, allowing a reduction of the possible diffusion of bacteria from the gut ($<$3 h is suggested by Winkelmayer et al. *(50)*).

To evaluate the suitability of the slaughtering process, the data for TVC and *Enterobacteriaceae* were compared with the process hygiene criteria set by EC Regulation No 2073/2005 *(16)* for cattle carcasses. For TVC, all the sampling sessions gave mean log values below the higher threshold (7 of 10 acceptable and the other 3 satisfactory). For *Enterobacteriaceae,* only two sampling sessions gave an unsatisfactory result. These data should be positively considered, as the thresholds are usually applied on the carcasses of relatively clean animals (the slaughtering of unacceptable dirty animals is not allowed by the Regulation EU No 853/2004 *(17),* whereas the hide contamination of wild boars cannot be avoided).

The correlation between bacterial numbers on the carcasses and the presence of target microorganisms was explored. Increases in *Listeria* spp. and *Campylobacter* spp. prevalences were observed when the TVC increased. Regarding *Campylobacter* spp., a prevalence of 9% was detected on carcasses, with TVC $<$ 3 log CFU/cm^2, whereas this rate increased to 23 and 25% when the TVC was

between 3 and 4 or $>$4 log CFU/cm^2, respectively. The prevalence of *Listeria* spp. was 18, 31, and 50% on carcasses with TVC $<$ 3, between 3 and 4, and $>$4 log CFU/cm^2, respectively. The evaluation of *Enterobacteriaceae* did not reveal any influence on the prevalence of the selected microorganisms.

The different influencing factors were then analyzed. TVCs detected on the carcasses were not significantly influenced by the sex, age, or weight of the animals. Nevertheless, enteric bacteria loads were clearly influenced by these factors. The carcasses from adult animals had significantly higher counts of *Enterobacteriaceae* ($P = 0.03$) and *E. coli* ($P = 0.04$) than lighter animals, confirming the significantly ($P = 0.03$) higher loads detected in heavier ($>$90-kg) animals. These results could be because of a more difficult management of heavy animals (e.g., recovery from the hunting place, transport), especially during slaughtering procedures (e.g., skinning of old animals with thick winter fur), as reported by previous studies *(1, 3, 8, 40)*. The sex of the animals significantly influenced the bacterial counts: significantly higher loads of *Enterobacteriaceae* ($P = 0.02$) and *E. coli* ($P < 0.01$) were detected in males than in females, confirming the observed trends, as males reached the highest weights.

The environmental temperature at the time of hunting had a clear influence on TVC, with significantly higher values ($P < 0.01$) when the temperature was above 15°C. These loads decreased during the hunting season from October to December. Our results confirmed the data obtained by other studies *(1, 40)*.

The results of the present study confirm the potential role of the wild boars as reservoirs for some pathogens (mainly *Campylobacter,* but also *L. monocytogenes* and *Salmonella*). Wild boar is currently one of the most widespread ungulate species in Italy; thus, boar meat could

have a role in the introduction of pathogens into consumers' kitchens. The data show good carcasses hygiene status, with generally acceptable contamination levels, and the absence of *Salmonella* and *L. monocytogenes*. The relatively low presence of *Campylobacter* spp. can be further limited by the freezing of meat, a practice that is often done by the hunters. Considering microbiological indicators, the application of good manufacturing practices is crucial in this particular situation, wherein industrial equipment is not easily used. The higher bacterial loads detected on the carcasses of old, heavy male boars highlights the role of a careful application of hygienic procedures.

The results stress the importance of the training of hunters on the proper management of the carcasses and on slaughtering procedures that can ensure the production of hygienic meat intended for self-consumption or local marketing.

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