Title

Factors affecting the microbiological load of Italian hunted wild boar meat (*Sus scrofa*)

Authors/affiliations

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Abstract

This study investigates the microbiological conditions before maturation of wild boar meat (*Sus scrofa*) processed in approved game handling establishments in Italy. Fillets and legquarters of 37 carcasses were tested to assess Aerobic Colony Count (ACC), *Enterobacteriaceae* Count (EC) and *Salmonella* presence. *Salmonella* was never found and mean values of ACC and EC were 4.67±1.78 SD and 2.60±1.58 SD log2 CFU/cm², respectively. Both ACC and EC increased with time between evisceration and skinning, were significantly higher in fillets and when meat was processed by untrained operators. ACC also increased with boars’ weight and when carcasses were cleaned with running potable water. Based on limits set by EU Regulation No 1441/2007 for pork meat, most legquarters resulted satisfactory or acceptable (59% for ACC and 70% for EC), while most fillets were unsatisfactory (76% ACC, 78% EC). Results show that the wild game meat supply chain can be a safe process when handling practices reported in European and National regulations are met.

Keywords

Game meat; boar carcasses; food safety; microbiological contamination; wild boar

Highlight:

- Legquarters microbiological load was mostly within the EU limits set for pork meat
- None of the samples showed *Salmonella* contamination
- Washing with running water worsens the microbiological quality of carcasses
- Microbial contamination increases with time between evisceration and skinning
- Correct training of operators reduces microbial contamination
Introduction

During the last twenty years the ungulate population of Italian northern Apennines, in particular wild boar (*Sus scrofa*) and roe deer (*Capreolus capreolus*), increased exponentially (Carnevali *et al.*, 2009). This demographic explosion is mainly due to the gradual desertion by human populations of rural and marginal areas in favour of the big residential areas. Moreover, during the last thirty years, the Apennines protected areas system was greatly enhanced, giving wildlife important refuge areas that are crucial during the hunting season.

The dramatic growth of wild ungulate populations has led to coexistence problems with humans (e.g. car strikes, crops and forestry damages, conservation issues, sanitary issues), but wild game meat consumption could contribute to convert this problem into a resource. Nowadays, wild ungulate meat is a well-known resource in some European countries like Germany, Austria and Scotland (Ramanzin *et al.*, 2010; Winkelmayer & Paulsen, 2008). Game meat can be imported, farmed or hunted. Italy imports game meat especially from Germany, Austria, Hungary and New Zealand (ISMEA, 2004 – Statistiche del Settore Carne; Pellicioli & Viganò, 2013). Game meat farms in Italy are not well represented and continue to have a negative trend after the initial success of the 80s (Carnevali *et al.*, 2009). Game meat hunting and consumption in Italy has been increasing along with wild ungulate population in the last 30 years and, for instance, in the hunting season 2009/10 more than 230,000 culled ungulates were estimated (Ramanzin *et al.*, 2010). The per capita yearly consumption of game meat in Italy is estimated to be 0.1-0.3 kg, depending on the region, but it becomes higher when it refers to hunters: in this case the consumption can reach 1.0-6.4 kg (Ramanzin *et al.*, 2010). Among Italian hunters wild boar (*Sus scrofa*) is the most consumed wild mammal species, followed by hare (*Lepus europaeus*) and roe deer (*Capreolus capreolus*) (Ferri *et al.*, 2017).

Wild ungulate meat is a food rich in nutrients with low fat contents, a correct polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) ratio, a right PUFA ω6/ω3 proportion and a good conjugated linoleic acid content (Ramanzin *et al.*, 2010; Secchiari *et al.*, 2001; Summer *et al.*, 1997; Zomborszky *et al.*, 1996). Wild boar meat in particular was compared to domestic pig meat, resulting less fat and with a better PUFA/SFA ratio (Barbani *et al.*, 2011; Marsico *et al.*, 2007; Ramanzin *et al.*, 2010; Sales & Kotrba, 2013), and in addition to physical activity diet is considered the main factor inducing this difference: these species are monogastric, so the assumption of fatty acids is directly linked to their concentration in muscles and adipose tissue (Nürnberg *et al.*, 1998; Wood *et al.*, 2008).

Nowadays, consumers nutritional trends are moving towards quality, safety and traceability, especially in regard to food of animal origin. If correctly processed, game meat could perfectly match all these requirements. Another important issue for food market today is the concept of organic food, along with local or “zero-mileage” food: the consumer is more willing to promote sustainable food products and local economies (Hoffman & Wiklund, 2006) and when obtained through local hunting or culling activity, game
meat gathers both sustainability and local economy issues. Finally, nowadays consumers are becoming increasingly aware of animal welfare in farming practice and awareness towards the ethical value of game meat is increasing. Intensive meat production, even when in line with the strict EU legislation, is perceived as ethically unpleasant by many consumers. On the contrary, game animals are born in the wild and live a free life, they feed in natural conditions and they can fulfil all the species’ physiological and ethological needs (Bruckner, 2007). Safety requirements of wild game meat in the EU are addressed by EU Regulations No 178/2002, 852/2004, 853/2004 and 854/2004. These regulations rule responsibilities, traceability and game meat safety, ensuring the same level of control granted for domestic animal meat. Some specific steps are particularly stressed out as they are crucial considering the origin of this type of meat. For instance, time between shooting and evisceration must be as short as possible. After a trained person has evaluated the absence of macroscopic lesions, the carcass must be carried as soon as possible to a handling establishment where it must be cooled at a maximum of 7°C. Skinned carcasses must be stored separately from not-skinned ones and skinning must be accurate, with knives changed or washed frequently; it is also fundamental to avoid contamination of muscles with residuals of the gastrointestinal tract possibly left in the abdomen, and, at the same time, prevent the contamination through skin and fur. Washing the carcass with water is not recommended by food hygiene regulations as it could spread bacteria and help their growth acting as a pabulum. Post-mortem official veterinary inspection of the carcass is required in order to declare the meat suitable to human consumption and marketable. Training of food business operators is considered essential by all regulations.

Nevertheless, some critical steps are not addressed by these regulations, even if they are considered fundamental in ensuring safety and quality of hunted game meat. Hunting methods can differ widely among species and regions, but only a few studies deal with the effects of the different techniques on stress experienced by the animals, and consequently on meat quality (Ramanzin et al., 2010).

The aim of the present study is to quantify the microbiological load of hunted wild boar meat in relation to its processing in approved game handling establishments in the northern Apennines of Italy. Results will be compared with EU Regulation No 1441/2007 criteria, which only relates to domestic animals, in order to suggest valid references for further updates.

Materials and methods

Wild boar (Sus scrofa) was the chosen species as it represents the most common wild ungulate in the northern Apennines. Sampling took place between January and April 2015. The samples were collected at two different game handling establishments, both in Bologna province (Emilia-Romagna Region, northern Italy). The animals came from Bologna and Parma province and they were all free-living individuals, shot down during culling or wildlife control activity.
3.1 Sampling

Samples were collected from a total of thirty-seven wild boars. Thirty-five (95%) carcasses were collected among wildlife control plans in protected areas (Parks and Natural Reserves) and two (5%) were collected by culling activities. For each carcass the following data were recorded: age, sex, area of origin, shot location, total and dressed weight, time interval between shooting and evisceration and between evisceration and skinning, cleaning with running potable water, training of operators of the establishment during previous samplings. Further information about the sample is provided in Table 1.

Sampling was run on two meat cuts: fillet (psoas major muscle) and legquarter (gluteus medius, gluteus maximus and semitendinosus muscle). These cuts were selected in order to evaluate if evisceration and skinning were correctly carried out, respectively. This approach is different from EU Regulation No 1261441/2007, which suggest a unique sampling at four different anatomical areas according to the different species to reflect the general hygiene of the carcass. Samples were collected according to ISO 17640:2003 and EU Regulation No 1441/2007, using non-destructive method swabbing, in the time between skinning and maturation. For each sampling, a kit including a 4x8 cm sterile dehydrated sponge and a sterile test tube containing 10 ml of Peptone Salt solution (produced by IZSLER from single components: Peptone-Biokar; Sodium chloride-Panreac) was used. The kits were kept refrigerated at 4°C during transportation. At sampling, the diluent was added to the sponge into the sterile bag and then the sponge was rubbed onto the sampling point, covering an area of 100 cm² through 10 vertical and 10 horizontal movements as recommended by EU Regulation No 1441/2007. For every carcass, each of the two meat cuts was sampled using two different sponges, one for bacterial enumeration (Aerobic Colony Count and Enterobacteriaceae Count) and one for Salmonella detection. After each sampling the sponges were sealed in their original bags, immediately refrigerated and delivered to the laboratory within 24 hours.

1393.2. Microbiological analysis

All samples were processed at the Istituto Zooprofilattico Sperimentale of Emilia-Romagna and Lombardia (IZSLER) in Bologna for Aerobic Colony Count (ACC), Enterobacteriaceae Count (EC) and Salmonella spp. detection. ACC was performed according to ISO 4833-2:2013/Cor1: 2014 and EC according to ISO 21528-134:2004. Salmonella detection was carried out with PCR Real Time according to AFNOR BRD 07/06 – 07/04. This method is validated following NF EN ISO 16140 (2003) by AFNOR for all human and animal food products and for production environment samples by comparison to the reference method NF EN ISO 6579 and its amendment. In case of positive PCR results these are confirmed by ISO 6579:2002/Cor 114.
1481:2004. All these methods are recommended by EU Regulation No 1441/2007 for the process hygiene evaluation of domestic ungulates meat production.

150

153.3 Statistical analysis

152 Variation in ACC and EC in wild boar meat was analysed through General Linear Mixed Models (GLMMs) with normal error distribution. In both models, counts (UCF/cm$^3$) were log-transformed (log10) prior to analysis to meet the assumption of normality, and boar’s ID and meat-processing establishment were included as random factors to account for repeated measures and potential variability between establishments, respectively. We explored the effect on bacterial loads of boar sex, total weight, meat cut (i.e. fillet or legquarter), time interval between shooting and evisceration (hereafter time SE), time interval between evisceration and skinning (hereafter time ES), shot location (i.e. abdomen, thorax or head), eventual cleaning with running potable water (i.e. yes/no) and eventual training of operators during previous samplings (i.e. yes/no). We first fitted full models including all the above-mentioned variables and their second order interactions and then obtained minimal models through stepwise selection based on AICc values. Significance level is set at 0.05 and data are presented as mean ± SE unless otherwise specified.

164 The analysis were carried out using PROC GLIMMIX in SAS 9.4 software (Copyright © 2012, SAS Institute Inc., Cary, NC, USA).

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167.4 Results

168 Aerobic Colony Count (ACC) mean value on the whole sample was 4.67 log UCF/cm$^2$ (95%CI: 4.26 – 5.08) and Enterobacteriaceae Count (EC) was 2.60 log UCF/cm$^2$ (95%CI: 2.23 – 2.96), but both were on average higher on fillets than on legquarters (Table 2). Salmonella was not detected in any of the samples analyzed.

171 ACC varied with cleaning, boar’s total weight, time ES and time ES by meat cut and by cleaning (Table 3). Independently from the meat cut, ACC was affected by cleaning, with carcasses that had been cleaned with running potable water showing significantly higher bacterial counts (5.12 ± 0.23 log UCF/cm$^3$; n=32) than uncleaned carcasses (4.08 ± 0.34 log UCF/cm$^3$; n=42) (Figure 1). ACC also increased with boars’ total weight and time ES (Table 3). However, the effect of time depended on both the meat cut and cleaning process. In carcasses that had not been cleaned with running potable water, and therefore had lower mean initial bacterial counts, the magnitude of the ACC increase per unit of time was greater (Figure 2). Additionally, the effect of time on ACC was greater on fillets compared to legquarters (Figure 3). EC significantly increased with time ES as well, but also varied depending on meat cuts and operators’ training (Table 3). Bacterial counts were significantly higher on fillets (3.5 ± 0.24 log UCF/cm$^3$; n=37) than legquarters (1.69 ±
1820.17 log UCF/cm$^2$; n=37), and before (3.11 ± 0.31 log UCF/cm$^2$; n=22) than after training (2.38 ± 0.22 log 183UCF/cm$^2$; n=52) (Figure 4).

1845. Discussion

185 Neither the EU nor Italian regulation set microbiological load limits for game meat, however our study 186showed ACC and EC mean values overall consistent with values reported in literature for wild boars (Gill, 1882007; Paulsen & Winkelmayer, 2004) and with the limits for domestic animals mentioned in the EU 189Regulation No 1441/2007. In this Regulation three different types of results are considered: satisfactory, 190acceptable and unsatisfactory. All mean results between "m" and "M" are considered acceptable, all mean 191results below "m" are considered satisfactory (Table 4). It should be noted that in the present study a non- 192destructive sampling was used, while values reported by the EU Regulation No 1441/2007 (i.e. 5 log 193UFC/cm$^2$ and 3 log UFC/cm$^2$, respectively) refer to destructive sampling. It is therefore necessary to apply 194the correction factor mentioned at paragraph 5 of Regulation guidelines (rep. N. 93/CPR of 10/05/2007). By 195applying the correction, ACC and EC limit values become, respectively, 4.3 log UFC/cm$^2$ and 2.3 log 196UFC/cm$^2$. It should also be considered that the limits set by the regulation are based on a single sampling of 197different cuts and considers the daily mean result of a whole establishment, instead of mean values of a 198single carcass. Considering all the samples irrespective of the meat cut, the total mean ACC and EC values 199obtained in the present study (cfr. Table 2) are only slightly above the limits indicated by the EU Regulation 200for domestic pig meat, thus suggesting good hygiene carcass management in the game handling 201establishments involved. The comparison with the regulation is particularly satisfying for mean legquarter 202values which were lower than the limits indicated for pork meat. Moreover, even considering single ACC 203and EC values obtained on each carcass, more than a half of our legquarter samples falls within satisfactory 204or acceptable quality classes (Table 4). Conversely, mean ACC and EC values for fillet were both higher than 205the EU limits indicated for pork meat, and most of the samples were classified as unsatisfactory (Table 4), 206thus showing not completely correct evisceration practices.

207Concerning the factors influencing differences in microbiological contamination, first of all we found that 208ACC increased with boars' total weight. Since boars' weight increases with age, this result suggests that 209older animals may be more contaminated than younger ones and that their carcasses should be therefore 210managed with particular care. Washing with running potable water contributed to an increase of ACC 211values over the limits set by the EU Regulation No 1441/2007, thus affecting the microbiological quality of 212the carcasses. On the contrary, ACC values in boar carcasses which were not washed resulted to be lower 213than the regulation limits. This cleaning practice is to discourage as it promotes spreading of ubiquitous 214bacteria all over the carcass and bacterial growth in different areas of the carcass. It is therefore important 215to train operators to handle and manage the carcasses correctly since the beginning of its processing, 216without the need to remedy contaminations later with ineffective methods.
Time between shooting and evisceration (Time SE) was excluded from both minimal models, as it was not significantly related to the microbiological condition of the carcasses. This was probably due to the fact that only 5% of the animals (2/37) were eviscerated later than 3h after shooting, which is the time range considered to be critical in limiting the bacterial spread from the gastro-intestinal tract (Avagnina et al., 2012).

On the contrary, a longer time span between evisceration and skinning (Time ES) showed the impact of the initial carcass contamination for both ACC and EC loads. This was not surprising, as the presence of fur is known to enhance the risk of carcass contamination (Casoli et al., 2005). However, the effect of time ES on ACC was significantly higher on fillet than legquarter. A possible explanation for this observed difference is that between evisceration and skinning the skin of the thigh remains intact keeping the legquarter muscles protected against external bacterial contamination. Conversely, the abdomen is open and exposed to generalist bacteria present on the fur and the skin, which can easily reach the abdominal walls for proximity. Further studies are needed to provide additional evidence.

ACC increased with time ES both in carcasses that had been cleaned and not cleaned with running potable water, reaching a plateau of about 5 log UCF/cm³ around 160 hrs post-evisceration. However, such increase was faster on carcasses that had not been cleaned with water, as their initial bacterial load was on average lower.

Finally, as expected EC values were significantly lower in legquarters than in fillets, with a mean value lower than the acceptable limit indicated in EU Regulation No 1441/2007. Additionally, training of operators induced a significant decrease in EC values and data suggest that this effect was more pronounced on legquarters than on fillet. This observation confirms the importance of operators' training, especially with regard to reducing faecal contamination during carcass dressing. In particular, the low legquarter microbiological load is an indicator of skinning process quality, which was always carried out in the establishments and never by hunters in the field. Our findings confirm that wild ungulates conferral in approved game handling establishments is to be implemented as it ensures a good carcass processing and a safer final product, especially if a continuous and appropriate training of the operators is carried out.

Salmonella was never found in any of the samples, as required by EU Regulation No 1441/2007 for meat of domestic ungulates. This represents a positive finding, since the pathogen was isolated from wild boar meat by other Italian (Decastelli et al., 1995; Rodas et al., 2014) and foreign studies (Kanai et al., 1997).

6. Conclusions

Our study shows that the wild game meat supply chain is a safe process when handling practices reported in European and National regulations are met. Bacterial contamination of wild boar meat was frequently lower than the limits set by EU Regulation No 1441/2007 for slaughtered domestic pigs. In particular, the levels of microbial contamination of legquarter cuts demonstrates that good hygienic practices were
followed by the operators in the game handling establishments. Fillets ACC an EC levels show instead that further improvements are necessary for the evisceration practices.

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Acknowledgements

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References


Table 1. Mean total and dressed weight of wild boar carcasses by sex, with indication on the shot location (i.e. number of animals shot in the chest, head or abdomen) and carcass processing (i.e. number of carcasses cleaned or not cleaned with running potable water)

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>Total Weight (Kg)</th>
<th>Dressed Weight (Kg)</th>
<th>Shot location (n)</th>
<th>Cleaning (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>95% CI</td>
<td>Mean</td>
<td>95% CI</td>
</tr>
<tr>
<td>Males</td>
<td>22</td>
<td>58.5</td>
<td>49.0 – 67.9</td>
<td>42.7</td>
<td>34.3 – 51.0</td>
</tr>
<tr>
<td>Females</td>
<td>15</td>
<td>56.2</td>
<td>43.0 – 69.4</td>
<td>41.9</td>
<td>31.5 – 52.2</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>57.6</td>
<td>50.2 – 64.9</td>
<td>42.3</td>
<td>36.2 – 48.5</td>
</tr>
</tbody>
</table>

Table 2. ACC and EC mean values (log UCF/cm$^3$) observed in different meat cuts of wild boars, with Standard Deviation (SD) and 95% Confidence Interval (CI)

<table>
<thead>
<tr>
<th>Meat cut</th>
<th>N</th>
<th>ACC (log UCF/cm$^3$)</th>
<th>EC (log UCF/cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Fillet</td>
<td>37</td>
<td>5.58</td>
<td>1.53</td>
</tr>
<tr>
<td>Legquarter</td>
<td>37</td>
<td>3.76</td>
<td>1.54</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>4.67</td>
<td>1.78</td>
</tr>
</tbody>
</table>

Table 3. Minimal models explaining variation observed in ACC and EC values (log UCF/cm$^3$) from wild boar meat (n=74).

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Source of variation</th>
<th>Parameter estimate ± SE</th>
<th>F</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>Meat cut</td>
<td>Fillet</td>
<td>0.73 ± 0.5</td>
<td>2.5</td>
<td>1, 66</td>
</tr>
<tr>
<td></td>
<td>Cleaning</td>
<td>Yes</td>
<td>2.5 ± 0.6</td>
<td>15.4</td>
<td>1, 66</td>
</tr>
<tr>
<td></td>
<td>Total weight</td>
<td></td>
<td>0.016 ± 0.006</td>
<td>6.4</td>
<td>1, 66</td>
</tr>
<tr>
<td></td>
<td>Time ES</td>
<td></td>
<td>0.017 ± 0.004</td>
<td>34.2</td>
<td>1, 66</td>
</tr>
<tr>
<td></td>
<td>Time ES by Cut</td>
<td>Fillet</td>
<td>0.014 ± 0.005</td>
<td>7.9</td>
<td>1, 66</td>
</tr>
<tr>
<td></td>
<td>Time ES by Cleaning</td>
<td>Yes</td>
<td>-0.014 ± 0.006</td>
<td>6.11</td>
<td>1, 66</td>
</tr>
<tr>
<td>EC</td>
<td>Meat Cut</td>
<td>Fillet</td>
<td>1.8 ± 0.3</td>
<td>45.6</td>
<td>1, 69</td>
</tr>
<tr>
<td></td>
<td>Training</td>
<td>No</td>
<td>1.05 ± 0.5</td>
<td>11.8</td>
<td>1, 69</td>
</tr>
<tr>
<td></td>
<td>Time ES</td>
<td></td>
<td>0.01 ± 0.003</td>
<td>13.35</td>
<td>1, 69</td>
</tr>
</tbody>
</table>
Table 4. Proportion of wild boar meat samples of satisfactory (< m), acceptable (m – M) or unsatisfactory (> M) quality based on ACC and EC limits defined by EU Regulation No 1441/2007 for non-destructive sampling in pork meat.

<table>
<thead>
<tr>
<th>Meat cut</th>
<th>ACC</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; m†</td>
<td>m - M</td>
</tr>
<tr>
<td>Fillet</td>
<td>11%</td>
<td>13%</td>
</tr>
<tr>
<td>Legquarter</td>
<td>43%</td>
<td>16%</td>
</tr>
<tr>
<td>Total</td>
<td>27%</td>
<td>15%</td>
</tr>
</tbody>
</table>

† ACC: 3.3 log UCF/cm³; EC: 1.3 log UCF/cm³

* ACC: 4.3 log UCF/cm³; EC: 2.3 log UCF/cm³