

**1Title**

2Factors affecting the microbiological load of Italian hunted wild boar meat (*Sus scrofa*)

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18

## 19Abstract

20This study investigates the microbiological conditions before maturation of wild boar meat (*Sus scrofa*)  
21processed in approved game handling establishments in Italy. Fillets and legquarters of 37 carcasses were  
22tested to assess Aerobic Colony Count (ACC), *Enterobacteriaceae* Count (EC) and *Salmonella* presence.  
23*Salmonella* was never found and mean values of ACC and EC were  $4.67 \pm 1.78$  SD and  $2.60 \pm 1.58$  SD log  
24CFU/cm<sup>2</sup>, respectively. Both ACC and EC increased with time between evisceration and skinning, were  
25significantly higher in fillets and when meat was processed by untrained operators. ACC also increased with  
26boars' weight and when carcasses were cleaned with running potable water. Based on limits set by EU  
27Regulation No 1441/2007 for pork meat, most legquarters resulted satisfactory or acceptable (59% for ACC  
28and 70% for EC), while most fillets were unsatisfactory (76% ACC, 78% EC). Results show that the wild game  
29meat supply chain can be a safe process when handling practices reported in European and National  
30regulations are met.

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## 32Keywords

33Game meat; boar carcasses; food safety; microbiological contamination; wild boar

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## 36Highlight:

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

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## 451. Introduction

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

52 The dramatic growth of wild ungulate populations has led to coexistence problems with humans (e.g. car  
 53 strikes, crops and forestry damages, conservation issues, sanitary issues), but wild game meat consumption  
 54 could contribute to convert this problem into a resource. Nowadays, wild ungulate meat is a well-known  
 55 resource in some European countries like Germany, Austria and Scotland (Ramanzin *et al.*, 2010;  
 56 Winkelmayr & Paulsen, 2008). Game meat can be imported, farmed or hunted. Italy imports game meat  
 57 especially from Germany, Austria, Hungary and New Zealand (ISMEA, 2004 – Statistiche del Settore Carne;  
 58 Pelliccioli & Viganò, 2013). Game meat farms in Italy are not well represented and continue to have a  
 59 negative trend after the initial success of the 80s (Carnevali *et al.*, 2009). Game meat hunting and  
 60 consumption in Italy has been increasing along with wild ungulate population in the last 30 years and, for  
 61 instance, in the hunting season 2009/10 more than 230,000 culled ungulates were estimated (Ramanzin *et*  
 62 *al.*, 2010). The *per capita* yearly consumption of game meat in Italy is estimated to be 0.1-0.3 kg, depending  
 63 on the region, but it becomes higher when it refers to hunters: in this case the consumption can reach 1.0-  
 64 4.0 kg (Ramanzin *et al.*, 2010). Among Italian hunters wild boar (*Sus scrofa*) is the most consumed wild  
 65 mammal species, followed by hare (*Lepus europaeus*) and roe deer (*Capreolus capreolus*) (Ferri *et al.*,  
 66 2017).

67 Wild ungulate meat is a food rich in nutrients with low fat contents, a correct polyunsaturated fatty acids  
 68 (PUFA) and saturated fatty acids (SFA) ratio, a right PUFA  $\omega 6/ \omega 3$  proportion and a good conjugated linoleic  
 69 acid content (Ramanzin *et al.*, 2010; Secchiari *et al.*, 2001; Summer *et al.*, 1997; Zomborszky *et al.*, 1996).  
 70 Wild boar meat in particular was compared to domestic pig meat, resulting less fat and with a better PUFA/  
 71 SFA ratio (Barbani *et al.*, 2011; Marsico *et al.*, 2007; Ramanzin *et al.*, 2010; Sales & Kotrba, 2013), and in  
 72 addition to physical activity diet is considered the main factor inducing this difference: these species are  
 73 monogastric, so the assumption of fatty acids is directly linked to their concentration in muscles and  
 74 adipose tissue (Nürnberg *et al.*, 1998; Wood *et al.*, 2008).

75 Nowadays, consumers nutritional trends are moving towards quality, safety and traceability, especially in  
 76 regard to food of animal origin. If correctly processed, game meat could perfectly match all these  
 77 requirements. Another important issue for food market today is the concept of organic food, along with  
 78 local or “zero-mileage” food: the consumer is more willing to promote sustainable food products and local  
 79 economies (Hoffman & Wiklund, 2006) and when obtained through local hunting or culling activity, game

80meat gathers both sustainability and local economy issues. Finally, nowadays consumers are becoming  
81increasingly aware of animal welfare in farming practice and awareness towards the ethical value of game  
82meat is increasing. Intensive meat production, even when in line with the strict EU legislation, is perceived  
83as ethically unpleasant by many consumers. On the contrary, game animals are born in the wild and live a  
84free life, they feed in natural conditions and they can fulfil all the species' physiological and ethological  
85needs (Bruckner, 2007). Safety requirements of wild game meat in the EU are addressed by EU Regulations  
86No 178/2002, 852/2004, 853/2004 and 854/2004. These regulations rule responsibilities, traceability and  
87game meat safety, ensuring the same level of control granted for domestic animal meat. Some specific  
88steps are particularly stressed out as they are crucial considering the origin of this type of meat. For  
89instance, time between shooting and evisceration must be as short as possible. After a trained person has  
90evaluated the absence of macroscopic lesions, the carcass must be carried as soon as possible to a handling  
91establishment where it must be cooled at a maximum of 7°C. Skinned carcasses must be stored separately  
92from not-skinned ones and skinning must be accurate, with knives changed or washed frequently; it is also  
93fundamental to avoid contamination of muscles with residuals of the gastrointestinal tract possibly left in  
94the abdomen, and, at the same time, prevent the contamination through skin and fur. Washing the carcass  
95with water is not recommended by food hygiene regulations as it could spread bacteria and help their  
96growth acting as a *pabulum*. *Post-mortem* official veterinary inspection of the carcass is required in order  
97to declare the meat suitable to human consumption and marketable. Training of food business operators is  
98considered essential by all regulations.

99Nevertheless, some critical steps are not addressed by these regulations, even if they are considered  
100fundamental in ensuring safety and quality of hunted game meat. Hunting methods can differ widely  
101among species and regions, but only a few studies deal with the effects of the different techniques on  
102stress experienced by the animals, and consequently on meat quality (Ramanzin *et al.*, 2010).

103The aim of the present study is to quantify the microbiological load of hunted wild boar meat in relation to  
104its processing in approved game handling establishments in the northern Apennines of Italy. Results will be  
105compared with EU Regulation No 1441/2007 criteria, which only relates to domestic animals, in order to  
106suggest valid references for further updates.

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### 1083. Materials and methods

109Wild boar (*Sus scrofa*) was the chosen species as it represents the most common wild ungulate in the  
110northern Apennines. Sampling took place between January and April 2015. The samples were collected at  
111two different game handling establishments, both in Bologna province (Emilia-Romagna Region, northern  
112Italy). The animals came from Bologna and Parma province and they were all free-living individuals, shot  
113down during culling or wildlife control activity.

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### 1153.1 Sampling

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117Samples were collected from a total of thirty-seven wild boars. Thirty-five (95%) carcasses were collected  
118among wildlife control plans in protected areas (Parks and Natural Reserves) and two (5%) were collected  
119by culling activities. For each carcass the following data were recorded: age, sex, area of origin, shot  
120location, total and dressed weight, time interval between shooting and evisceration and between  
121evisceration and skinning, cleaning with running potable water, training of operators of the establishment  
122during previous samplings. Further information about the sample is provided in Table 1.

123Sampling was run on two meat cuts: fillet (*psoas major* muscle) and legquarter (*gluteus medius*, *gluteus*  
124*maximus* and *semitendinosus* muscle). These cuts were selected in order to evaluate if evisceration and  
125skinning 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  
127species to reflect the general hygiene of the carcass. Samples were collected according to ISO 17640:2003  
128and EU Regulation No 1441/2007, using non-destructive method swabbing, in the time between skinning  
129and maturation. For each sampling, a kit including a 4x8 cm sterile dehydrated sponge and a sterile test  
130tube containing 10 ml of Peptone Salt solution (produced by IZSLER from single components: Peptone-  
131Biokar; Sodium chloride-Panreac) was used. The kits were kept refrigerated at 4°C during transportation. At  
132sampling, the diluent was added to the sponge into the sterile bag and then the sponge was rubbed onto  
133the sampling point, covering an area of 100 cm<sup>2</sup> through 10 vertical and 10 horizontal movements as  
134recommended by EU Regulation No 1441/2007. For every carcass, each of the two meat cuts was sampled  
135using two different sponges, one for bacterial enumeration (Aerobic Colony Count and *Enterobacteriaceae*  
136Count) and one for *Salmonella* detection. After each sampling the sponges were sealed in their original  
137bags, immediately refrigerated and delivered to the laboratory within 24 hours.

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### 1393.2. Microbiological analysis

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141All samples were processed at the Istituto Zooprofilattico Sperimentale of Emilia-Romagna and Lombardia  
142(IZSLER) in Bologna for Aerobic Colony Count (ACC), *Enterobacteriaceae* Count (EC) and *Salmonella* spp.  
143detection. ACC was performed according to ISO 4833-2:2013/Cor1: 2014 and EC according to ISO 21528-  
1442:2004. *Salmonella* detection was carried out with PCR Real Time according to AFNOR BRD 07/06 – 07/04.  
145This method is validated following NF EN ISO 16140 (2003) by AFNOR for all human and animal food  
146products and for production environment samples by comparison to the reference method NF EN ISO 6579  
147(2002) and its amendment. In case of positive PCR results these are confirmed by ISO 6579:2002/Cor

1481:2004. All these methods are recommended by EU Regulation No 1441/2007 for the process hygiene  
149evaluation of domestic ungulates meat production.

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### 1513.3 Statistical analysis

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153Variation in ACC and EC in wild boar meat was analysed through General Linear Mixed Models (GLMMs)  
154with normal error distribution. In both models, counts (UCF/cm<sup>3</sup>) were log-transformed (log<sub>10</sub>) prior to  
155analysis to meet the assumption of normality, and boar's ID and meat-processing establishment were  
156included as random factors to account for repeated measures and potential variability between  
157establishments, respectively. We explored the effect on bacterial loads of boar sex, total weight, meat cut  
158(*i.e.* fillet or legquarter), time interval between shooting and evisceration (hereafter time SE), time interval  
159between evisceration and skinning (hereafter time ES), shot location (*i.e.* abdomen, thorax or head),  
160eventual cleaning with running potable water (*i.e.* yes/no) and eventual training of operators during  
161previous samplings (*i.e.* yes/no). We first fitted full models including all the above-mentioned variables and  
162their second order interactions and then obtained minimal models through stepwise selection based on  
163AICc values. Significance level is set at 0.05 and data are presented as mean ± SE unless otherwise specified.  
164The analysis were carried out using PROC GLIMMIX in SAS 9.4 software (Copyright © 2012, SAS Institute  
165Inc., Cary, NC, USA).

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### 1674. Results

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169Aerobic Colony Count (ACC) mean value on the whole sample was 4.67 log UFC/cm<sup>2</sup> (95%CI: 4.26 – 5.08)  
170and *Enterobacteriaceae* Count (EC) was 2.60 log UFC/cm<sup>2</sup> (95%CI: 2.23 – 2.96), but both were on average  
171higher on fillets than on legquarters (Table 2). *Salmonella* was not detected in any of the samples analyzed.

172ACC varied with cleaning, boar's total weight, time ES and time ES by meat cut and by cleaning (Table 3).  
173Independently from the meat cut, ACC was affected by cleaning, with carcasses that had been cleaned with  
174running potable water showing significantly higher bacterial counts ( $5.12 \pm 0.23$  log UCF/cm<sup>3</sup>; n=32) than  
175uncleaned carcasses ( $4.08 \pm 0.34$  log UCF/cm<sup>3</sup>; n=42) (Figure 1). ACC also increased with boars' total weight  
176and time ES (Table 3). However, the effect of time depended on both the meat cut and cleaning process. In  
177carcasses that had not been cleaned with running potable water, and therefore had lower mean initial  
178bacterial counts, the magnitude of the ACC increase per unit of time was greater (Figure 2). Additionally,  
179the effect of time on ACC was greater on fillets compared to legquarters (Figure 3). EC significantly  
180increased with time ES as well, but also varied depending on meat cuts and operators' training (Table 3).  
181Bacterial counts were significantly higher on fillets ( $3.5 \pm 0.24$  log UCF/cm<sup>3</sup>; n=37) than legquarters ( $1.69 \pm$

1820.17 log UCF/cm<sup>3</sup>; n=37), and before ( $3.11 \pm 0.31$  log UCF/cm<sup>3</sup>; n=22) than after training ( $2.38 \pm 0.22$  log  
183UCF/cm<sup>3</sup>; n=52) (Figure 4).

#### 1845. Discussion

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186Neither the EU nor Italian regulation set microbiological load limits for game meat, however our study  
187showed ACC and EC mean values overall consistent with values reported in literature for wild boars (Gill,  
1882007; Paulsen & Winkelmayr, 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<sup>2</sup> and 3 log UFC/cm<sup>2</sup>, 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<sup>2</sup> and 2.3 log  
196UFC/cm<sup>2</sup>. 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.

217Time between shooting and evisceration (Time SE) was excluded from both minimal models, as it was not  
218significantly related to the microbiological condition of the carcasses. This was probably due to the fact that  
219only 5% of the animals (2/37) were eviscerated later than 3h after shooting, which is the time range  
220considered to be critical in limiting the bacterial spread from the gastro-intestinal tract (Avagnina *et al.*,  
2212012).

222On the contrary, a longer time span between evisceration and skinning (Time ES) showed the impact of the  
223initial carcass contamination for both ACC and EC loads. This was not surprising, as the presence of fur is  
224known to enhance the risk of carcass contamination (Casoli *et al.*, 2005). However, the effect of time ES on  
225ACC was significantly higher on fillet than legquarter. A possible explanation for this observed difference is  
226that between evisceration and skinning the skin of the thigh remains intact keeping the legquarter muscles  
227protected against external bacterial contamination. Conversely, the abdomen is open and exposed to  
228generalist bacteria present on the fur and the skin, which can easily reach the abdominal walls for  
229proximity. Further studies are needed to provide additional evidence.

230ACC increased with time ES both in carcasses that had been cleaned and not cleaned with running potable  
231water, reaching a *plateau* of about 5 log UCF/cm<sup>3</sup> around 160 hrs post-evisceration. However, such increase  
232was faster on carcasses that had not been cleaned with water, as their initial bacterial load was on average  
233lower.

234Finally, as expected EC values were significantly lower in legquarters than in fillets, with a mean value lower  
235than the acceptable limit indicated in EU Regulation No 1441/2007. Additionally, training of operators  
236induced a significant decrease in EC values and data suggest that this effect was more pronounced on  
237legquarters than on fillet. This observation confirms the importance of operators' training, especially with  
238regard to reducing faecal contamination during carcass dressing. In particular, the low legquarter  
239microbiological load is an indicator of skinning process quality, which was always carried out in the  
240establishments and never by hunters in the field. Our findings confirm that wild ungulates conferral in  
241approved game handling establishments is to be implemented as it ensures a good carcass processing and a  
242safer final product, especially if a continuous and appropriate training of the operators is carried out.

243*Salmonella* was never found in any of the samples, as required by EU Regulation No 1441/2007 for meat of  
244domestic ungulates. This represents a positive finding, since the pathogen was isolated from wild boar  
245meat by other Italian (Decastelli *et al.*, 1995; Rodas *et al.*, 2014) and foreign studies (Kanai *et al.*, 1997).

246

## 247 **6. Conclusions**

248Our study shows that the wild game meat supply chain is a safe process when handling practices reported  
249in European and National regulations are met. Bacterial contamination of wild boar meat was frequently  
250lower than the limits set by EU Regulation No 1441/2007 for slaughtered domestic pigs. In particular, the  
251levels of microbial contamination of legquarter cuts demonstrates that good hygienic practices were



252 followed by the operators in the game handling establishments. Fillets ACC an EC levels show instead that  
 253 further improvements are necessary for the evisceration practices.

254

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

Sex	N	Total Weight (Kg)		Dressed Weight (Kg)		Shot location (n)			Cleaning (n)	
		Mean	95% CI	Mean	95% CI	Chest	Head	Abd.	Yes	No
Males	22	58.5	49.0 – 67.9	42.7	34.3 – 51.0	13	7	2	13	9
Females	15	56.2	43.0 – 69.4	41.9	31.5 – 52.2	9	2	4	8	7
<b>Total</b>	<b>37</b>	<b>57.6</b>	<b>50.2 – 64.9</b>	<b>42.3</b>	<b>36.2 – 48.5</b>	<b>22</b>	<b>9</b>	<b>6</b>	<b>21</b>	<b>16</b>

335

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

Meat cut	N	ACC (log UCF/cm <sup>3</sup> )			EC (log UCF/cm <sup>3</sup> )		
		Mean	SD	95% CI	Mean	SD	95% CI
Fillet	37	5.58	1.53	5.07 – 6.09	3.50	1.48	3.00 – 4.00
Legquarter	37	3.76	1.54	3.25 – 4.28	1.69	1.06	1.34 – 2.05
<b>Total</b>	<b>74</b>	<b>4.67</b>	<b>1.78</b>	<b>4.26 – 5.08</b>	<b>2.60</b>	<b>1.58</b>	<b>2.23 – 2.96</b>

338

339

340**Table 3. Minimal models explaining variation observed in ACC and EC values (log UCF/cm<sup>3</sup>) from wild**  
 341**boar meat (n=74).**

Response variable	Source of variation		Parameter estimate ± SE	F	df	p
<b>ACC</b>	Meat cut	Fillet	0.73 ± 0.5	2.5	1, 66	0.12
	Cleaning	Yes	2.5 ± 0.6	15.4	1, 66	0.0002
	Total weight		0.016 ± 0.006	6.4	1, 66	0.014
	Time ES		0.017 ± 0.004	34.2	1, 66	<0.0001
	Time ES by Cut	Fillet	0.014 ± 0.005	7.9	1, 66	0.0063
	Time ES by Cleaning	Yes	-0.014 ± 0.006	6.11	1, 66	0.016
<b>EC</b>	Meat Cut	Fillet	1.8 ± 0.3	45.6	1, 69	<0.0001
	Training	No	1.05 ± 0.5	11.8	1, 69	0.001
	Time ES		0.01 ± 0.003	13.35	1, 69	0.0005

342

343 **Table 4. Proportion of wild boar meat samples of satisfactory (< m), acceptable (m - M) or unsatisfactory**  
 344 **(> M) quality based on ACC and EC limits defined by EU Regulation No 1441/2007 for non-destructive**  
 345 **sampling in pork meat.**

Meat cut	ACC			EC		
	< m†	m - M	> M*	< m†	m - M	> M*
Fillet	11%	13%	76%	11%	11%	78%
Legquarter	43%	16%	41%	51%	19%	30%
<b>Total</b>	27%	15%	58%	31%	15%	54%

346† ACC: 3.3 log UCF/cm<sup>3</sup>; EC: 1.3 log UCF/cm<sup>3</sup>

347\* ACC: 4.3 log UCF/cm<sup>3</sup>; EC: 2.3 log UCF/cm<sup>3</sup>

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