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The microbiological conditions of carcasses from large game animals in Italy

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Abstract

This study investigates the microbiological conditions of large game animal carcasses following evisceration. Carcasses of animals (N = 291) hunted in the Upper Susa Valley (Italian Alps) were analysed for pH, Aerobic Viable Count (AVC), *Enterobacteriaceae*, *Yersinia* spp., *Listeria monocytogenes* and *Salmonella* spp. After shooting, evisceration occurred within 60 min in 90.7% of animals and sampling within 90 min in 88.3% of animals. Mean pH values (5.97: ruminants; 5.77: wild boar) were similar to those of regularly slaughtered domestic species. AVC values were highest in animals shot in the abdomen. Within species, AVC and *Enterobacteriaceae* values did not differ across different shooting-evisceration/sampling times. However, these counts exceeded 5 and 2.5 log, respectively, in 18% of wild boar and 39% of ruminants; the highest values were detected in wild boar. No pathogens were detected in any species. These results reveal inadequate hygiene in game meat handling/harvesting, implicating the need for improved practices.

Keywords: Game meat; Hunting; Microbiological quality; Food safety

1. Introduction

Game meat is a traditional food in Northern Italy, with consumption per capita being 4 kg/year in hunter families (Ramanzin et al., 2010). This trend has been linked to an excessive growth of wild ungulate populations in many areas, therefore requiring management strategies to limit the expansion of such populations (Côté et al., 2004).

Various studies (e.g. Atanassova et al., 2008, Eglezos et al., 2008, Gill, 2007 and Paulsen and Winkelmayer, 2004) have investigated the hygienic quality of game meat, considering both microbiological criteria (Aerobic Viable Count [AVC], Enterobacteriaceae) and the presence of pathogenic bacteria (*Listeria monocytogenes*, *Salmonella* spp. and *Yersinia enterocolitica*). However, such studies on game hunted in European countries are lacking in the literature, and the majority of the published articles have focused on the analysis of meat obtained from red deer, roe deer and wild boar. The aim of the present investigation was to analyse a larger number of game meat samples – arising from four different species hunted within the Italian Alps – compared to other internationally relevant Italian studies (Decastelli, Giaccone, & Mignone, 1995). In addition, this study specifically aimed to collect data with regard to chamois meat, in light of its large population size in Italy and the traditional hunting of this species within Alpine regions. Previous studies have investigated various geographic areas, but this is the first to focus exclusively on game meat shot in the Alps, which represents the major group of mountains in Europe. Thus, to fill this gap in the literature, the present investigation selectively collected data from a specific hunting district within the Western Italian Alps (the Upper Susa Valley) where the hunting of these species has been commonplace for more than 20 years. The selected hunting district contains a collection point that permits the acquisition of large amounts of data on diverse large game species. By limiting this investigation to a select geographical zone, the data obtained will also be relevant for many other, geographically similar, hunting districts within the Alps, as well as other mountainous areas. A further objective of this research was to provide the important data necessary for the development and validation of game meat risk assessment models to be applied throughout Europe (Membré, Laroche, & Magras, 2011).

2. Material and methods

2.1. Carcass sampling

Samples were collected from the following species hunted within the Upper Susa Valley Hunting district in the Western Alps (total area: 64 119 ha; altitude: 600–2700 m): chamois (*Rupicapra rupicapra*), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*); between the months of September and December, for three consecutive years (2008–2010). In 2008 census data for this area estimated the presence of 1643 chamois, 936 red deer and 1226 roe deer; no census data were available for wild boar. A large and modern collection point is situated within the area and all hunters are obliged to present their shot animals for the mandatory collection of morphometric data: horn length, state of teeth eruption and leg length, as well as animal gender. These data are required by the hunting district in order to verify the hunters' compliance with the specified hunting rules.

The hunting of roe deer, chamois and red deer is only allowed by rifle (minimum calibre, 6 mm or 0.243 in.) by spot and stalk hunting. While wild boar, on the other hand, are usually driven-hunted

with hounds and killed using shotguns (12 gauge slugs, as buckshot is not authorised in Italy). After killing and dressing (in the field), hunters were required to transport animals to the collection point for the taking of biometric measurements; thereafter, carcasses are not held at the station, but are carried away by the hunters, who perform chilling, skinning and cutting on their own premises. It is important to emphasize that following the usual harvesting procedures, large game is kept chilled skin-on for several days.

All samples were collected from animals on their arrival at the collection point, and hunter participation in the survey was on a voluntary basis only. For each tested carcass, a data sheet was compiled, which recorded: body area hit by bullet(s), calibre of rifle, time elapsed between shooting and evisceration, and time elapsed between evisceration and sampling/pH measurement. For the purpose of analysis, data were divided into groups defined by the amount of time elapsed between shooting and evisceration (< 30 min; 30 to 60 min; 61 to 180 min; > 180 min), and between shooting and sampling (< 60 min; 61 to 90 min, 91 to 180 min; > 180 min).

At the collection station, the surface pH of the muscles in the medial part of the hindlimb region (semitendinosus/semimembranosus) was measured using a pH metre equipped with a Xerolyte electrode (CRISON Model 507 pH metre). pH measurements were categorised as originating from either ruminant or wild boar carcasses and analysed separately; mean pH and 95% confidence intervals (95% C.I.) were calculated. Finally, at the collection point (and, therefore, prior to chilling), four samples of each carcass were collected by swabbing a 25 cm2 area on the surface of the muscles within the anatomical region of the medial hindlimb. This sampling technique was preferred over the excision method in order to preserve the anatomical integrity of carcasses. Indeed, since samples were provided by hunters on a voluntary basis, it was important to avoid the incurrence of any significant economical loss for the participant, particularly considering the small carcass sizes of the hunted animals.

For AVC and *Enterobacteriaceae* enumeration, swabs were transferred into 10 ml of peptone saline solution (8.5 g NaCl, 1.5 g Peptone, 1000 ml H2O). For the detection of Salmonella spp., samples were pre-enriched in Buffered Peptone Water (BPW) (CM 509 B, Oxoid — Rodano, Milan); for the detection of Listeria spp., samples were pre-enriched in half Fraser Broth (TN 1034, SIFIN — Berlin); and for the detection of Yersinia spp., samples were pre-enriched in Phosphate Buffered Saline (PBS) (8.08 g Na2HPO4, 1.37 g KH2PO4, 8.5 g NaCl, 1000 ml H2O). All samples were stored at 4 °C and analyses performed the day after sample collection.

2.2. Enumeration of AVC and *Enterobacteriaceae*

For AVC and *Enterobacteriaceae* counts, the respective procedures were followed: ISO 4833 (2004) and AFNOR NF V08-054 (1999).

2.3. Detection of pathogenic bacteria

The isolation of *Salmonella* spp. was carried out in accordance with ISO 6579 (1993). In brief, following the pre-enrichment of samples in BPW for 24 h at 37 °C, 0.1 ml and 1 ml of each pre-enrichment solution were inoculated into 10 ml of Selenite Cystine Broth base (CM 0699, Oxoid — Rodano, Milan) and 10 ml of Rappaport-Vassiliadis Broth (CM 669 B, Oxoid — Rodano, Milan), and then incubated at either 37 °C (Selenite Cystine Broth) or 42 °C (Rappaport-Vassiliadis Broth)

for 24 h, and then plated onto selective Xylose Lysine Deoxycholate (XLD) Agar (CM 0469, Oxoid — Rodano, Milan). Following 24 h incubation at 37 °C, suspect colonies of Salmonella spp. were tested using API 20 Enterobacteriaceae (API 20E) strips (BioMérieux Italia — Bagno a Ripoli, Florence).

For the detection of *L. monocytogenes*, the ISO 11290–1:1996/Amd 1:2004 (2004) procedure was followed. In brief, following incubation of each sample in Half Fraser Broth (TN 1034, SIFIN - Berlin) at 30 °C for 24 ± 2 h, 100 µl of the sample/pre-enrichment solution was inoculated into 10 ml of Fraser Broth (TN 1035, SIFIN — Berlin) and then incubated at 37 °C for 24 ± 2 h. The enrichment broth was then streaked onto Oxoid Chromogenic Listeria Agar (OCLA; CM 1080, Oxoid — Rodano, Milan), and selective PALCAM Agar (CM 0877 B, Oxoid — Rodano, Milan). Colonies with a typical L. monocytogenes appearance were identified using a species-specific Polymerase Chain Reaction (PCR) (D'Agostino et al., 2004).

Yersinia spp. was detected using a previously published protocol (Niskanen, Waldenström, Fredriksson-Ahomaa, Olsen, & Korkeala, 2003) to which minor modifications were made. In brief, following 24 h in PBS at 4 °C, 1 ml was streaked directly onto Cefsulodin–Irgasan–Novobiocin (CIN) Agar medium (CM 653 B, Oxoid — Rodano, Milan) (day 0). In parallel, samples were also cold pre-enriched in PBS at 4 °C for 1, 7, 14, and 21 days. On each of the respective day, samples were alkali treated (1 ml of each sample added to 5 ml of an alkaline solution: 5 g NaCl, 2.5 g KOH in 1000 ml distilled water) for 3 min in order to increase the selectivity of the medium and then streaked onto CIN Agar (CM 653 B, Oxoid — Rodano, Milan). After plating, samples were incubated at 31 °C for 24 h. Suspected *Yersinia* colonies were subcultured in Kligler Iron Agar (KIA) (CM0033, Oxoid — Rodano, Milan) and further tested using motility-test media (BALL-SELLERS, Biolife Italiana S.r.l. — Milan) and API 20E (BioMérieux Italia — Bagno a Ripoli, Florence). Moreover, in order to confirm isolates as *Yersinia* spp. and to investigate the pathogenic potential of the isolates, a specific PCR targeting inv, ail and yst genes was carried out, as described by Falcão et al. (2004).

2.4. Statistical analyses

Samples from a total of 291 game carcasses were collected; however, samples from 40 animals were excluded from the study because of logistical problems, leaving 251 samples for bacteriological analysis. Statistical analyses considered all 251 samples and were performed using SAS® v. 9.1 software for windows (SAS Institute, Cary, North Carolina). The results of the statistical tests were considered significant for $p < 0.05$ and highly significant for $p < 0.01$.

2.4.1. Species differences: ruminants vs. wild boar

AVC and *Enterobacteriaceae* counts were compared for ruminants (N = 186) vs. wild boar (N = 65) using the two-sample Wilcoxon test. This non-parametric test was selected because of the high frequency of samples providing counts below the detection limit of the microbiological method used (20% for both AVC and *Enterobacteriaceae*).

2.4.2. Influence of shooting-evisceration and shooting-sampling times

Since hygienic parameters may be influenced by the time elapsed between shooting and evisceration and between shooting and sampling, AVC and *Enterobacteriaceae* data were divided

into groups according to the shooting-evisceration and shooting-sampling time ranges and the groups compared using the non-parametric Kruskal–Wallis test. Analyses were performed for each species separately. In order to investigate further the differences in the distribution of the AVC and *Enterobacteriaceae* data across the different time ranges between wild boars and the ruminant species, an association analysis was performed using the χ2 test.

2.4.3. Influence of anatomical shooting location

In view of the fact that carcass bacterial load could be affected by shooting location, AVC and *Enterobacteriaceae* counts were compared between animals shot in the abdominal region (any location posterior to the diaphragm, N = 99) and all other locations (animals shot in the head or neck, heart, or spine, N = 152) using the non-parametric two-sample Wilcoxon test. In order to explore the effect of species, an association analysis (using the χ2 test) was performed to investigate abdominal shooting differences across species.

2.4.4. *L. monocytogenes* and *Yersinia* spp. frequency difference across species

Differences in the frequencies of *L. monocytogenes* and *Yersinia* spp. between species were investigated using the χ2 test. For *Yersinia* spp. a sample was considered positive when the microorganism was detected in at least one of the five analysed time points: day 0 and following 1, 7, 14 and 21 days of incubation.

3. Results

3.1. Carcass sampling

Samples from a total of 291 animals were collected: chamois (N = 85), roe deer (N = 78), red deer (N = 56) and wild boar (N = 72). Samples were collected across three different hunting seasons: 26% of samples in 2008, 43% in 2009 and 31% in 2010. All animals were eviscerated in the field, the majority of which (90.7%) occurred within 60 min after shooting; specifically, 58.1% were eviscerated within 30 min after killing and 32.6% between 31 min and 1 h. The remaining 9.3% of the carcasses were eviscerated at a later time (between 61 min and 3 h after the shooting). Samples were collected on the arrival of the carcasses at the collection point (between 1 and 6 h after shooting): the majority of the samples (88.3%) were collected not later than 90 min after killing.

In order to conduct further analyses, animals were divided into those shot in the abdominal cavity (anywhere below the diaphragm) (39.4%) and those shot elsewhere (60.6%): heart (38.7%), head or neck (17.1%), and spine (4.8%).

3.2. pH measurement

The pH measurements obtained between 30 min and 6 h after the killing demonstrated the ruminant carcasses (N = 219) to be characterised by a mean pH of 5.97 (95% C.I.: 5.92–6.02), whereas those of wild boar (N = 72) were characterised by a mean pH value of 5.77 (95% C.I.: 5.69–5.85).

3.3. AVC and *Enterobacteriaceae* enumeration

3.3.1. Species differences: ruminants vs. wild boar

The AVC and the *Enterobacteriaceae* counts for the different species are reported in Table 1. When the AVC and *Enterobacteriaceae* counts were compared between ruminants vs. wild boar (Wilcoxon two-sample test), highly significant differences in the median log-values between the two groups were identified (AVC: z = 6.35 log cfu/cm2, p < 0.01; *Enterobacteriaceae*: z = 4.70 log cfu/cm2, p < 0.01). The AVC median log-values in wild boar were higher than those of ruminants: 4.61 log cfu/cm2 vs. 3.30 log cfu/cm2, respectively. Similarly, *Enterobacteriaceae* counts were significantly higher in the wild boar: 3.00 log cfu/cm2 in wild boar vs. 1.80 log cfu/cm2 in ruminants.

3.3.2. Influence of shooting-evisceration and shooting-sampling times

No significant differences were found for AVC and *Enterobacteriaceae* counts compared within each species across the shooting-evisceration and shooting-sampling time groups. Similarly, no significant differences between were found between the data frequency distributions across time ranges for wild boar vs. ruminant species.

3.3.3. Influence of anatomical shooting location

The frequencies of animal shot in the abdomen were similar for all the species: 39% in chamois, 49% in roe deer, 25% in red deer and 43% in wild boar. However, a significant difference was detected when AVC counts were compared between animals shot in the abdominal cavity and those shot elsewhere (AVC: z = 2.02 log cfu/cm2, p < 0.05), indicating that the AVC median log-value was significantly higher when animals were shot in the abdominal region (3.98 log cfu/cm2) than when shot elsewhere (3.56 log cfu/cm2).

3.4. Detection of bacterial pathogens

L. monocytogenes was recovered from 7 out of 251 samples analysed (2.8%) and *Listeria ivanovii* in only 1 sample (collected from a chamois). A total of 28 *Yersinia* spp. were isolated from 18 out of 251 animals (7.2%): 10 wild boar (15.4%), 4 red deer (7.1%), 3 roe deer (4.9%) and 1 chamois (1.5%). The identification of these isolates was confirmed by means of API 20E (Biomerieux Italia — Bagno a Ripoli, Florence), and 6 of these were identified as *Y. enterocolitica* species with a likelihood level greater than 90%. These 6 isolates were retrieved from 1 chamois, 1 roe deer, 1 red deer and 3 wild boars. PCR results showed that all 6 strains showed the presence of the *inv* gene, whereas ail and *yst* genes were never amplified.

The analysis of *L. monocytogenes* and *Yersinia* spp. frequencies across species revealed significant differences only for *Yersinia* spp ($\chi2 = 8.82$), with a significantly higher detection rate in wild boar than in the other three species (p < 0.05).

4. Discussion

The aim of the present study was to investigate the hygienic quality of game meat from wild ungulates hunted in an Italian Alpine area (altitude range: 600–2700 m above sea level) by collecting relevant microbiological data. This is the first Italian study to collect large amounts of microbiological data from large game carcasses arising exclusively from an Alpine hunting district.

4.1. pH

As the shooting of the animals took place in different geographical locations within the hunting district and the carcasses were not subsequently stored at the collection point, it was not possible to standardise a specific time point for the measurement of the muscle surface pH of the medial hindlimb. Nevertheless, pH measurements taken between 30 min and 6 h of animal killing were not found to vary considerably from those registered for domestic species. Mean muscle pH was found to be lower in the wild boar (5.77) than in the ruminant species (5.97). These results may indicate that the harvesting practices do not modify the natural early acidification processes of the carcasses.

4.2. Aerobic viable and Enterobacteriaceae counts

4.2.1. Species differences: ruminants vs. wild boar

Highly significant differences were observed in AVC and *Enterobacteriaceae* counts between ruminants and wild boar. These differences may be related to differences between species and/or shooting/evisceration times and shooting/sampling times, which may increase microbial loads (Koréneková & Korénekov, 2008). Therefore, the distributions of AVC and *Enterobacteriaceae* data for wild boar and ruminants were compared across 4 different time ranges. The results did not reveal any significant differences in frequency between wild boar and ruminants, implicating that the significantly higher AVC and *Enterobacteriaceae* counts observed in wild boar are most probably related to hunting and/or handling methods which differ between game species. Indeed, within the hunting district considered, wild boar hunters use shotguns and stalk or drive animals with hounds, while the ruminant species considered (roe deer, red deer and chamois) are only hunted by rifle (minimum calibre, 6 mm or 0.243 in.) by spot and stalk hunting. The use of lead slug in shotguns for wild boar (usually Brenneke slugs, characterised by a poor terminal ballistic performance) may produce wounds which are often not fatal, allowing animals to keep running and thus increasing contamination levels (Atanassova et al., 2008). The high contamination loads revealed in wild boar carcasses may also be related to meat cross-contamination from microorganisms present in the skin, considering that wild boar skin is more severely contaminated than that of ruminants due to ethological differences (Atanassova et al., 2008). Finally, these observations confirm that a continuous and strict cold chain should be maintained in game meat storage in order to prevent rapid spoilage of the meat (Paulsen & Winkelmayer, 2004).

As regards the three ruminant species (roe deer, red deer and chamois), despite differences in the habitats of these three species, the hygienic quality of the game meat was similar. With regard to chamois, the results revealed the median AVC counts to be similar to those of the other ruminant species, but the frequency of carcasses presenting counts above 5 log cfu/cm2 was lower, even considering that animals were shot in geographical locations which rendered carcass recovery difficult. This observation may be related to the fact that chamois hunting occurs above altitudes of 1000 m, where weather conditions such as low temperatures and humidity may delay bacterial growth, thus explaining the fact that the frequency of *Enterobacteriaceae* counts below the detection threshold was the highest for this species.

4.3. Influence of shooting-evisceration and shooting-sampling times

We hypothesised that bacterial counts might be influenced by the time elapsed between shooting and evisceration/sampling, in particular considering that the selected hunting area may pose specific problems for hunts, which could significantly delay the recovery of the carcasses, evisceration and the transport of the carcasses to the collection point where sampling took place. The statistical analyses revealed that AVC and *Enterobacteriaceae* counts in each species did not differ significantly across either shooting-evisceration or shooting-sampling time ranges. However, the lack of any statistical significance may be related to the grouping of the data into time categories which subsequently reduced the sample size of each group. The majority of animals were eviscerated within 1 h (90.7%) and samples taken within 90 min of shooting (88.3%). What is more, high levels of contamination were also recorded in animals eviscerated/sampled within 30–60 min after shooting, as well as in animals eviscerated/sampled more than 180 min after shooting; thus indicating that, besides the influence of the time elapsed between shooting-evisceration/sampling, handling and harvesting practices may also play an important role in determining carcass microbial loads in game meat. Furthermore, the absence of a significant correlation between time elapsed since shooting and microbiological condition of the meat may be due to the fact that less than 4% of the animals were eviscerated at a time later than 3 h after shooting — the time span considered to be critical for limiting the bacterial spread from the guts (Winkelmayer, Paulsen, Lebersorger, & Zedka, 2008).

4.4. Aerobic viable and Enterobacteriaceae counts — influence of shooting location

The AVC results of present study are consistent with those of Winkelmayer et al. (2008), who found that animals shot in any location posterior to the diaphragm (abdominal shootings) can be reasonably considered to be at higher risk of microbiological contamination than those shot anywhere else (heart, head and neck, spine). On the other hand, no statistical difference could be observed for the *Enterobacteriaceae* counts between animals shot in the abdominal region and those shot elsewhere, implying that these counts are not related to the location of the shooting, but are probably due to improper evisceration procedures.

4.5. Bacterial pathogens

In the present study, the detection of *Salmonella* spp., *Listeria* spp. and *Yersinia* spp. revealed low contamination frequency in game meat. The results for Salmonella spp. detection were in agreement with most of the published data which also showed low frequencies of this pathogen in venison (Atanassova et al., 2008, Paulsen and Winkelmayer, 2004 and Wahlström et al., 2003). However, in the case of wild boars, a higher Salmonella spp. isolation frequency was expected, given the wide range of positive sample frequencies reported in the literature (Atanassova et al., 2008 and Bensik et al., 1991). These discordant results may be related to Salmonella spp. frequency variation across populations (Gill, 2007). In particular, the data in this research suggest that the wild boar population living in the Western Italian Alps may harbour the pathogen at such a low frequency that a much larger sample size would be necessary to detect it.

The results of this study also show that chamois meat can be occasionally contaminated by *L. monocytogenes* and *L. ivanovi* at a frequency which is comparable with that of other hunted species. It should be highlighted here that the results obtained in this research could be attributed to the sampling method used, which swabbed a muscular area limited to 25 cm^2. However, the presence of

low frequencies of *Listeria* spp. have already been reported in the literature, and this microorganism is not strictly linked to animal hosts, but it can also form part of the microflora contaminating the environment (Atanassova et al., 2008, Paulsen and Winkelmayer, 2004 and Paulsen et al., 2003). With regard to *Yersinia* spp., very little data is available on game meat. Kanai et al. (1997) found that wild boar and red deer meats were positive for *Yersinia* spp. in approx. 40% and 30% of samples, respectively. In the present study, *Yersinia* spp. was isolated in all species even if a limited sample size was considered; the detection frequency was highest in wild boar meat whereas the prevalence in chamois was similar to that of roe and red deer. The higher detection of *Yersinia* spp. over *Y. enterocolitica* may suggest that meat contamination is related to environmental rather than faecal contamination of the carcasses. In fact, none of the tested strains harboured pathogenicity genes (such as *ail* and *yst*), suggesting that the presence of *Y. enterocolitica* on the surface of the sampled area (hindlimb muscle) may be due to contamination of the skin by non pathogenic bacterial strains. These strains may be present in the environment and able to reach the muscles after the animals are wounded or dragged after death, events which are more frequent in the hunting of wild boar (Atanassova et al., 2008).

Overall, the low frequency of *Listeria* spp. and *Yersinia* spp., and the absence of *Salmonella* spp. revealed in this study could be attributed to the particular geographical area being tested where animals were all free living species, seldom sharing pastures with domestic animals. In fact, in the Upper Susa Valley area, domestic livestock are only grazed in the mountainous areas during the summer (the so-called activity of "alpeggio"), therefore limiting the transmission of pathogens from intensively farmed animals to wild fauna (French, Rodriguez-Palacios, & Le Jeune, 2010).

4.6. Significance for food safety and quality

In order to formulate an assessment of the hygienic quality of game meat analysed, AVC and *Enterobacteriaceae* counts were compared with criteria specified by Regulation (EC) No. 1441/2007, (2007,7,12), which amends the Regulation (EC) No. 2073/2005 referred to by Atanassova et al. (2008). The EU regulation criteria were referred to for guidance only, as they refer to samples collected using the excision method, which could not be applied in this research for the previously discussed reasons. The incidence of AVC above the 5 log cfu/cm^2 limit set by the above mentioned EU Regulation occurred in 7–16% of ruminant species and in more than 30% of wild boars. The high microbial loads revealed in the present study emphasise that the quality of meat is highly dependent on the first hunting phases, and anatomical shooting location plays a critical role in carcass hygiene (Paulsen, Bauer, Winkelmayer, Smulders, & Hofbauer, 2005). These findings support the implementation of good hygienic procedures, particularly during the evisceration, transport, skinning and cutting operations (Atanassova et al., 2008 and Gill, 2007).

Interestingly, even if the literature reports that swabbing methods may lead to the recovery of low bacterial loads, in the present research relatively high counts were observed in comparison with those obtained in research where the excision method was used (Atanassova et al., 2008), thus indicating that the hygienic quality of game meat hunted in the Upper Susa Valley (Western Italian Alps) may be severely impaired. In fact, these high microbial loads were often associated with visible contamination of the carcass with soil or gut content and large openings of the body cavities (30% of all animals and 40% of roe deer). Furthermore, the observation of high microbial loads is consistent with that reported by Miraglia, Ranucci, D'Ovidio, Branciari, and Severini (2005), who

suggested that the swabbing of a small area (e.g. 25 cm^2) can lead to higher isolation rates, thus producing results comparable to those obtained by the excision method.

These observations strengthen the need for the rigorous application of good harvesting practices by hunters (who are identified by the EU Regulation as primary producers) and all those responsible for game meat quality, as well as the putting into effect of traceability requirements, as required by Regulation (EC) No. 853/2004 (2004,30,04) and (EC) No. 178/2002 (2002,1,2).

However, it needs to be emphasized that the proper training of hunters may be an unrealistic solution for improving the hygienic quality of game meat harvested in the Upper Susa Valley. In fact, some hunters are devoted to maintaining hunting traditions (e.g. removing visible contamination using water from small rivers or natural canals) which, although being deeply rooted in popular culture, are not based on acknowledgeable safety principles and may result in the impairment of game meat quality. Taking this consideration into account, it should be emphasized that more studies are needed on the proper handling and management of carcasses, in light of the influence of different environments and hunting techniques upon meat hygienic quality.

In conclusion, even though a considerable number of samples showed high AVC and *Enterobacteriaceae* counts, no significant levels of pathogenic bacteria were detected. These findings might indicate that game meat producers need to address spoilage and shelf life problems more than safety issues. However, considering that *Listeria* spp. and *Yersinia* spp. are able to grow at refrigeration temperatures, the recovery of these bacterial species from a limited number of samples may still represent a problem, mostly with respect to meat storage and processing. In fact, the microorganisms can contaminate the environment and cross contaminate other carcasses/foods, which may represent a risk particularly in some typical raw foods such as "carpaccio" or short-ripened sausages, which are consumed in Italian Alpine regions. This emphasizes the importance of proper handling in all phases of game production, from hunting to fork, in order to guarantee food that meets the EU quality and safety standards.

Finally, this survey may provide useful data for research efforts aimed at producing models on risk assessments for game meat throughout Europe (Membré et al., 2011). This is a major challenge considering the variability of wild fauna which populate the different geographic areas, the differences in hunting and processing practices, as well as the different pathogen prevalence in the wild population.

Acknowledgments

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Table 1

Median Log (cfu/cm) of microbial counts for each species. Each column shows the distribution of the number of animals by species and Log AVC and *Enterobacteriaceae* counts divided into three classes in light of legal criteria (Regulation (EC) No 1441/2007): the number of animals per class of microbial load is followed by numbers, in brackets, indicating the relative percentage (across columns).

Microbial load	Chamois (N = 69)		Roe deer (N = 61)		Red deer (N = 56)		Wild boars (N = 65)	
	AVC	*Enterobacteriaceae*	AVC	*Enterobacteriaceae*	AVC	*Enterobacteriaceae*	AVC	*Enterobacteriaceae*
Below detection limit[a]	3 (4.35)	27 (39.13)	1 (1.64)	16 (26.23)	0	20 (35.71)	0	8 (12.31)
Log (cfu/cm^2) $\leq 5^b$ (≤ 2.5)^c	61 (88.40)	20 (28.99)	51 (83.61)	14 (22.95)	47 (83.93)	23 (41.07)	44 (67.69)	25 (38.46)[d]
Log (cfu/cm^2) $> 5^b$ (> 2.5)^c	5 (7.25)	22 (31.88)	9 (14.75)	31 (50.82)	9 (16.07)	13 (23.21)	21 (32.31)	32 (49.23)[e]
Median log cfu/cm^2	3.23	1.30	3.46	2.47	3.31	1.70	4.61[d]	3.00[f]

[a]Detection limit: 10 cfu/cm^2; [b]limit for AVC; [c]limit for *Enterobacteriaceae*; [d]limit for wild boars set to ≤ 3 Log (cfu/cm^2); [e]limit for wild boars set to > 3 Log (cfu/cm^2); [f]Indicates statistical significance when wild boars counts were compared with those of ruminants.