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The microbiological conditions of carcasses from large game animals in Italy

A. Avagnina [a], D. Nucera [a,*], M.A. Grassi [a], E. Ferroglio [b], A. Dalmasso [a], T. Civera [a]

[a] Department of Animal Pathology, University of Turin, via Leonardo da Vinci 44, 10095 Grugliasco, TO, Italy
[b] Department of Animal Production, Epidemiology and Ecology, University of Turin, via Leonardo da Vinci 44, 10095 Grugliasco, TO, 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.

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, Apelt, Reich, & Klein, 2008; Eglezos, Dykes, Huang, Fegan, & Stuttard, 2008; Gill, 2007; Paulsen & 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: 64119 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

* Corresponding author. Tel.: +39 0116709334; fax: +39 0116709224.
E-mail address: daniele.nucera@unito.it (D. Nucera).

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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 cm^2 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 H_2O). 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 Na_2HPO_4, 1.37 g KH_2PO_4, 8.5 g NaCl, 1000 ml H_2O). 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 χ^2 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