Bacterial Contamination of Wild Boar (*Sus Scrofa)* Meat handled by Hunters, Local Retail Establishments and Game Handling Establishments in Sweden

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Bacterial Contamination of Wild Boar (*Sus Scrofa)* Meat Handled by Hunters, Local Retail Establishments and Game Handling Establishments in Sweden.

Bakteriell kontamination av vildsvinskött (*Sus Scrofa*) som hanterats av jägare, lokala detaljhandelsanläggningar och vilthanteringsanläggningar i Sverige.

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Abstract

As part of the Swedish food strategy, the government developed a proposal to enable hunters to sell small quantities of wild boar meat directly to consumers instead of via a registered game handling establishment. Whether this threatens food safety is debated. Thirty-nine samples of wild boar mince, where the carcass has been slaughtered and further handled throughout the processing chain by hunters (H) themselves, local retail establishments (LRE) or game handling establishments (GHE) were evaluated according to the hygienic criteria for minced meat in (EC) No 2073/2005 regulating *E. coli* and aerobic bacteria levels. *Enterobacteriaceae* were also analysed as hygiene indicators. All 17 GHE samples were unsatisfactory due to heightened aerobic levels, however, all but one had *E. coli* levels below or within the allowed span. Two of 11 H samples failed due to aerobic bacteria and two exceeded *E. coli* levels. Five LRE samples were unsatisfactory due to aerobic bacteria alone, and three samples exceeded both limits. In total, 29 samples were deemed *'unsatisfactory'*, 6 as '*acceptable'* and 4 as '*satisfactory*'. Average bacteria levels for *E. coli* were H= 2.1 log cfu/g, LRE= 2.4 log cfu/g and GHE= 2.1 log cfu/g. Average aerobic counts in H samples (6.1 log cfu/g) were significantly lower (p= 0.00003) than in LRE (7.3 log cfu/g) and GHE samples (7.7 log cfu/g). Genera recovered from the *Enterobacteriaceae* family, identified through MALDI-TOF, were *Escherichia, Yersinia* spp., *Pantoea, Serratia* spp., *Leclercia, Klebsiella* spp., *Buttiauxella, Pseudescherichia, Citrobacter, Hafnia* and *Kalamiella*. The results suggest that it is possible for hunter to produce safe meat, however, more improvements in hygiene are needed throughout the entire production chain.

Keywords: wild boar, slaughter hygiene, hygiene, wild game slaughter hygiene, local retail establishment, game handling establishment.

Sammanfattning

Som en del av den svenska livsmedelsstrategin har regeringen tagit fram ett förslag som gör det möjligt för jägare att sälja små mängder vildsvinskött direkt till konsument istället för via en registrerad vilthanteringsanläggning. Huruvida detta hotar livsmedelssäkerheten är omdiskuterat. Trettionio prover av vildsvinsfärs där slakt och produktion har gjorts av jägare (H), lokala detaljhandelsanläggningar (LRE) eller vilthanteringsanläggningar (GHE) utvärderades enligt de hygieniska kriterierna för färs i (EG) nr 2073/2005 som reglerar halterna av *E. coli* och aeroba bakterier. *Enterobacteriaceae* analyserades också som hygienindikatorer. Alla 17 GHE-prover var otillfredsställande på grund av förhöjda nivåer av aeroba bakterier, men alla utom ett hade *E. coli*-nivåer under eller inom det tillåtna intervallet. Två av 11 H-prover underkändes på grund av höga halter av aeroba bakterier och två överskred *E. coli*-nivåerna. Fem LRE-prover var otillfredsställande enbart på grund av aeroba bakterier, och tre prover överskred båda gränsvärdena. Totalt bedömdes 29 prover som "otillfredsställande", 6 som "acceptabla" och 4 som "tillfredsställande". Genomsnittliga bakterienivåer för *E. coli* var H = 2,1 log cfu/g, LRE = 2,4 log cfu/g och GHE = 2,1 log cfu/g. Det genomsnittliga antalet aeroba bakterier i H-proverna (6,1 log cfu/g) var signifikant lägre (p = 0,00003) än i LRE- (7,3 log cfu/g) och GHE-proverna (7,7 log cfu/g). De släkten från *Enterobacteriaceae*-familjen som påträffades i proverna, och identifierades genom MALDI-TOF, var *Escherichia, Yersinia* spp, *Pantoea, Serratia* spp, *Leclercia, Klebsiella* spp, *Buttiauxella*, *Pseudescherichia*, *Citrobacter, Hafnia* och *Kalamiella.* Resultaten tyder på att det är möjligt för jägare att producera säkert kött, men att det behövs fler förbättringar av hygienen genom hela produktionskedjan.

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Abbreviations

GHE	Game handling establishment, registered at the Swedish Food Agency
GMP	Good Manufacturing Program
H	Hunter samples
HACCP	Hazard Analysis of Critical Control Points
FBO	Food Business Operator
LRE	Local retail establishments as defined in (EC) No 853/2004 that has been incorrectly registered for wild boar meat handling, and unregistered (illegal) establishments with similar traits of that of an actual local retail establishment
PCA	Plate Count Agar, a general media for aerobic bacteria
SPW	Saline Peptone Water
TBX	Tryptone Bile X- glucuronide Agar, a selective and differential agar for coliforms and *Escherichia Coli*
VRBG	Violet Blue Bile Agar, a media used for enumeration of Enterobacteriaceae

1. Introduction

According to studies, the consumption of game meat has increased over the past years (Ljung *et al*., 2014; Sveriges Radio P4, 2021; Jordbruksverket, 2024a). It is appreciated due to its high culinary value (Lizana *et al.* 2022). This increase could be explained by a number of factors. For example, there is a cultural and social value connected to hunting, and game meat has a high nutritional profile (Corradini et al., 2022). Wild boar *(Sus Scrofa)* meat has a higher content of α-tocopherol which increases its shelf life, and it has a high mineral content (The Swedish Food Agency, 2019a; Lizana *et al.* 2022). Game meat also addresses environmental and ethical concerns raised in connection to industrial meat production (Corradini et al., 2022; World Wide Fund for Nature, 2023). However, the World Wide Fund for Nature also points out that it would not be practical, nor environmentally friendly, to increase game meat production to such an extent that it could replace the quantities of meat consumed today (World Wide Fund for Nature, 2023).

Wild boar was extinct from the Swedish fauna in the 1700s due to extensive hunting (Gren et al., 2019; Naturvårdsverket, 2020) before it was reintroduced through escapes from enclosures in the 1970s. Since then, the number of animals along with the harvest numbers has increased (Markon et al., 2022; Gren *et al.* 2019). In the hunting season of 1990-1991 approximately 350 wild boars were shot compared to the 112 700 animals that were harvested in the hunting season of 2022-2023 (Viltdata.se, 2023). A national survey conducted in 2023 indicated that around 20 % of the Swedish population eats game meat on a regular basis (Ipsos, 2023). An older study from 2014 reported similar numbers (around 22%) and noted that the highest consumption rates were documented in the northern parts of Sweden and the lowest in Stockholm County. The same survey also reported that about a third of all game meat consumed was derived from wild boar (Ljung et al., 2014). The majority of game meat has previously been sold as frozen products, but several game handling facilities and slaughterhouses have seen an increased demand for fresh and vacuum-packed meat over the past years (Naturvårdsverket, 2014).

Compared to other Swedish game species, the sale of wild boar meat is surrounded by stricter regulations (LIVSFS 2005:20). This is mainly because wild boars are known carriers of pathogens such as *Trichinella, Toxoplasma gondii, Salmonella* spp. and *Yersinia* spp. (The Swedish Food Agency, 2019a; Gomes-Neves *et al.* 2021). Therefore, there are limited ways for hunters to distribute their harvested wild boar meat today. The first option is to deliver the meat for further slaughter and examination at an approved game handling establishment (GHE, EC 853/2004). At these GHE the hunter is allowed to either sell the meat to the GHE for further distribution to a final consumer, or to collect the meat after slaughter and consume it within their own household or hunting team. It is also possible for the hunter to refrain from leaving the meat at an GHE and oversee slaughter, examination and processing themselves, provided that the meat is consumed within the own household or hunting team (The Swedish Food Agency, 2021).

A common way for game meat to reach consumers is through family members and friends, restaurant visits, stores, or local farm shops (Ljung *et al.* 2014). It is also relatively common for landowners that lease their hunting ground to hunters to include a 'meat clause' in the leasing contract, giving them rights to an agreed amount of meat (Naturvårdsverket, 2014). However, there is still a lot of wild boar meat that does not reach the general public (Jordbruksverket, 2024b). The Swedish Board of Agriculture (Jordbruksverket) estimate that around 2% of a person's total meat consumption per year consists of wild game meat (Jordbruksverket, 2023). Statistics from the Swedish Food Agency show that only a small amount, about 14 % per cent, of the meat from wild boar killed in Sweden reaches the market via a GHE (The Swedish Food Agency, 2019a). More recent calculations show that out of 14,900 tonnes of game meat, approximately 2700 tonnes, or 18%, were wild boar meat (Malmsten, 2023, pers. comm).

In relation to the increased interest in wild boar meat and as a part of the Swedish food strategy (Jordbruksverket, 2023; Prop. 2016/17:104), the Swedish government assigned the Swedish Food Agency to assess: a) the possibilities of simplifying the distribution of wild boar meat and facilitating its path to consumers; and b) how to implement this in a safe manner (Prop. 2016/17:104; Näringsdepartementet, 2020). This commission resulted in two reports named 'Avsättning av svenskt viltkött' [Distribution of Swedish game meat] and 'En jägares leveranser av små mängder vildsvin och kött av vildsvin' [A hunter's deliveries of small quantities of wild boar and wild boar meat] that were published in 2019 and 2021, respectively (The Swedish Food Agency, 2019a; The Swedish Food Agency, 2021).

In addition to the assessment from the Swedish Food Agency, the latter report also included views and opinions of the industry, hunters' organisations, and other authorities to act as a base for the development of new national regulations concerning the sales of wild boar meat to consumers and retailers. The regulations would authorise hunters to sell smaller amounts of wild boar meat, or unskinned carcasses of wild boar, directly to consumers, provided that they have passed two courses of study concerning slaughter hygiene: one dedicated to inspection of wild game, and one specific to wild boar meat handling and hygiene. Besides taking the courses, the hunter would also have to be registered at the County Administrative Board and its local municipality. If these premises were fulfilled, it would allow the hunter to examine, approve and sell wild boar meat themselves, thus bypassing the GHE. Another point included in the suggested measures was to allow hunters without an additional wild boar handling education to supply small amounts of wild boar meat to restaurants, public meal kitchens, and shops, assuming that the receiving businesses took on the responsibility of sampling the meat and handling the meat properly. Furthermore, these types of businesses would have to notify their control authority, usually the local municipality, that they would handle wild boar meat. Lastly, the suggestion would make it possible for registered LRE to sell meat directly to the final consumer under the conditions that they fulfilled certain requirements, such as having proper hygiene educations, having a special ID to trace products, and conducting *Trichinella* and cesium sampling if the wild boar has been harvested in areas where there is a risk of high cesium content in the animals (e.g. Västmanland, Uppsala and Gävleborg counties). The proposal is now under consideration by the European Commission and other Member States and has not yet been adopted (The Swedish Food Agency, 2021; LIVSFS 2024: XX.,2024).

Another type of establishment available on the market is a so called ‘local retail establishment (LRE) (EC 852/2004; The Swedish Food Agency, 2024d). Currently, these establishments are not allowed to slaughter nor sell wild boar meat to either hunters or other consumers, unless the meat has been examined at a GHE beforehand. The suggestion would make it possible for a registered LRE to handle and sell wild boar meat to a final consumer under the conditions that they fulfilled certain requirements such as having proper hygiene educations, having a special ID to trace their products, and conducting *Trichinella* and cesium sampling if the wild boar has been harvested in areas where there is a risk of high cesium content in the animals (e.g Västmanland, Uppsala and Gävleborg counties). The proposal is now under consideration by the European Commission and other Member States and has not yet been adopted (The Swedish Food Agency, 2021; LIVSF 2024: XX.,2024).

The (currently) illegal direct sale of wild boar meat is known to be quite extensive (Jordbruksverket, 2013; SVT NYHETER/ Halland, 2020) where one pathway of the wild boar meat is through the use of an unregistered (and thus illegal) local establishment. This type of establishment is in some ways similar to a LRE when it comes to facilities, equipment, and the possibility to keep carcasses and meat at sufficient temperatures. An unregistered local establishment is usually run by one or more persons, often with work experience from a commercial slaughterhouse (or similar). The establishment receives wild boar carcasses (or other carcasses from other game species) from hunters (skinned or unskinned) and provides a service that includes skinning, followed by cutting and packing the game meat, upon which the hunters later collect the packed meat for their own consumption. The service is paid for by the hunters. As these establishments are not registered, there are no records of how many that exist, nor is there any information on hygiene practices. In some instances, LREs are wrongfully registered to handle and process uninspected wild boar carcasses.

Reports of foodborne disease resulting from the consumption of game meat are virtually non-existent, according to reports from the Swedish Food Agency (The Swedish Food Agency, 2023a). This may be partly due to a low reporting of foodborne disease in general, that the suspected food has been discarded already, that only one person has become ill which does not count as an outbreak, or that the infected person/persons does not seek health care (The Public Health Agency of Sweden, 2016; The Swedish Food Agency, 2023a).

The purpose of the report is to gain a better understanding of potential contamination sources of wild boar meat by collecting data on bacterial contamination, including bacterial numbers and bacterial genera in minced wild boar meat collected from three groups: hunters, unauthorised or wrongfully registered LREs and GHEs. The data can also be used to get an overview of slaughter hygiene and potential sources of contamination of Swedish wild boar meat, as there are few previous studies treating the subject.

2. Purpose

The purpose of this report was to investigate and compare the microbiological quality of minced wild boar meat prepared by 1) game handling establishments, 2) unregistered local establishments as described in the introduction, and where wild boar carcasses are processed in facilities not provided by the hunter + wrongfully registered local retail establishments and 3) individual hunters. The work will also assess hygienic quality by comparing the bacterial levels to hygienic recommendations for minced meat in regulation (EC) No 2073/2005. As there are few currents reports on the subject comparing and investigating wild boar mince from the actors above, the results of the report can also be used to gain a better understanding of slaughter hygiene and potential sources of contamination of Swedish wild boar meat.

2.1 Demarcations

All samples were delivered and collected during the period of late January to May 2024, which will have affected the microbial growth rate due to a more rapid cooling of the carcasses, compared to seasons with warmer outside temperatures (Paulsen, 2011; Gomez-Neves *et al.* 2021; Lizana *et al.* 2022). No farmed game was examined.

Note, to facilitate the reading of the text and the results, unregistered/illegal establishments with ways similar to a registered LRE when it comes to facilities, equipment, and the possibility to keep carcasses and meat at sufficient temperatures, and wrongfully registered local retail establishments will still be referred to as local retail establishments (LRE) when presenting results and discussion.

Specific analyses to detect *Salmonella* were not performed as the sampling area was not marked as a designated risk zone according to the Swedish Veterinary Agency (SVA, 2024).

The legislated halt in the hunting period of wild boar (Jaktförordningen 1987:905) during the duration of the project led to practical constraints when collecting samples. Thus, the total number of samples varies within the three categories, namely, 11 samples from hunters, 11 samples from LRE (or similar), and 17 samples from GHE.

Literature used to compile this paper has been retrieved from scientific publications, legal texts and reports using the search engines ScienceDirect, Google Scholar, Wiley Online Library and Primo. Where necessary, facts have been gathered from organisations connected to the wild game supply chain.

3. Background

The section below will give a description of game handling establishments, how they are defined, and general hygiene procedures. A description concerning hygienic criteria for minced meat and common microbes found in wild boars, as well as a brief overview of the discussion regarding slaughter hygiene of wild boar meat will also be presented.

3.1 Game Handling Establishment (GHE)

For an establishment to be classified as a game handling establishment (GHE), it needs to be approved by the Swedish Food Agency. As of 2023, there were 182 approved GHEs in Sweden (The Swedish Food Agency, 2023c).

When establishing a GHE, the food business operator (FBO) applies at the Swedish Food Agency for approval of the GHE. The Food Agency will review the application and do an on-site inspection of the premises and the in-house control program. Such an inspection includes a review of the building and the equipment, as well as a review of the infrastructure to ensure that it allows a safe flow of meat through the production. It is of outmost importance that the FBO has identified and analysed critical hazards at the establishment, and how the HACCP (Hazard Analysis of the Critical Control Points) and GMP (Good Manufacturing Program) are supposed to mitigate these hazards (The Swedish Food Agency, 2024a).

A risk classification is also made for GHE and is based on the different activities planned to take place on-site. At the GHE, the carcass can be unskinned and stored until a *postmortem* inspection is performed. The activities such as cutting, production of minced meat or meat preparations and products thereof are to be approved separately because they are not included in a GHE approval. When a GHE have been approved, the establishment will receive an approval number to be able to assure traceability of products (The Swedish Food Agency, 2023b). This number is also used for health marking the carcasses at the GHE and serves as an identification mark for the products from possible cutting, minced meat and products from the other approved activities on the same site (The Swedish Food Agency, 2024d).

The FBO has the main responsibility for non-fraudulence and foods safety. Nevertheless, a GHE and other possible activities on site must comply, at minimum, to the general hygiene regulations of (EC) No 852/2004 and the specified regulations in (EC) No 853/2004 for food of animal origin (The Swedish Food Agency, 2024a). There are also other regulations regarding microbiological criteria (2073/2005), *Trichinella* spp. (2015/1375) and labelling (1169/2011) of the products.

A hunter can either deliver the wild game directly to a GHE, or to a collection centre for wild game registered at the county administrative board. If this collection centre is on-site at the GHE, approval is made by the Swedish Food Agency. Collection centres are only used for storage of wild game directly from hunters, while waiting for transport to the GHE for further handling (EC No 853/2004; The Swedish Food Agency, 2023g). The hunter must fulfil some criteria when delivering a carcass to a GHE. Firstly, the carcass must be delivered unskinned and secondly, organs such as the spleen, liver, heart, lungs, kidney, and skull must be sent along with the carcass for further examination. However, if a trained person (as defined in EC No 853/2004, Section IV, Chapter I.) has examined the carcass and organs, and concluded that there were no abnormalities noted, a certificate can be filled in and be sent along with the carcass to the collection centre or directly to the GHE. If a trained person has examined the carcass, no organs except the head and diaphragm from species susceptible to trichinosis must accompany the carcass to the GHE (EC No 853/2004; The Swedish Food Agency, 2023g).

If something unusual is noted the certificate cannot be signed. For example, if the hunter who examines the carcass detects any abnormalities in the animal's behaviour before the shot or on the carcass or organs, and nevertheless, decides to deliver the carcass to the GHA, the findings must be noted and communicated to the GHE. If a paper documenting the abnormalities is filled in, this must be sent to the GHE together with all the organs. However, if there are no anomalous findings on the abdominal organs, these can be left in the hunting ground. As the nature of the findings can vary a lot, it is important to communicate with the GHE, and if possible, the official control personnel at the GHE. Also, it must be noted that the specially trained person must have participated in the hunt when the game was shot or stay in direct connection to the hunting grounds to do an examination (The Swedish Food Agency, 2024b).

Upon arrival of the wild game, the FBO makes a first control and handling of the carcass. The *postmortem* inspection of the carcass and the organs is performed by an official veterinarian or auxiliary, and it must take place as soon as possible. If any certificates from the trained person are available, these are checked instead of the organs. At this point, the wild boar is also sampled for *Trichinella* (The Swedish Food Agency, 2024b). Health marking on the carcasses can be done first after acceptable results from *Trichinella* testing and, in some cases, also from Cesium-137 testing (The Swedish Food Agency, 2019b).

Some GHEs have implemented hygiene practices to increase carcass quality from start, by giving some guidelines to the hunters regarding slaughter hygiene on their websites. For example, a knife can be cleaned by using a cloth soaked in alcohol or vinegar. Furthermore, the hunters are also encouraged to deliver the wild game as soon as possible after killing and evisceration, especially if the outside temperature exceeds 10-12 ℃ (Gårdsvilt AB, 2023; Ekovilt, 2020; Skånska Kött och Vilt, 2024).

As previously mentioned, a GHE has certain hygiene requirements to fulfil. In addition to current regulations, there are also available industry guidelines for slaughterhouses. The latter are not compulsory but can be used to provide tips and advice on how to organise a slaughtering establishment to obtain safe foods (The Swedish Food Agency, 2023h**).** Also, the GHEs are compelled to keep unclean and clean process steps separated in time or space. An example of a clean area is where the carcasses are handled after skinning and the surface of the carcass is clean (The Swedish Food Agency, 2021; Laaksonen & Paulsen 2015).

3.2 Local Retail Establishments (LRE)

A local retail establishment (LRE) registered at the municipality is referred to in (EC) No 853/2004 and 22 § LIVSF 2005:20 and can be defined as an establishment that carries out retail activities involving the direct sale or supply of foods of animal origin to the consumer. One of criteria that must be met by a LRE is that the consumers/customers have to be located within the county to which the primary production area belongs, or in a neighbouring county (LIVSFS 2005:20). The second criterion that needs to be met is the production volume: a LRE is allowed to have a production volume of 500 kg of product per week. Besides local customers and a limited production volume, a LRE is required to offer a limited number of products or have a limited number of recipients. Currently, all wild game that is handled by a LRE has to be of species <u>other than</u> wild boar, bear, badger, and beaver. Thus, if these criteria are met, it is sufficient that the establishment complies with the general hygiene requirements of Regulation (EC) No 852/2004 and is registered at their local municipality where the establishment is located. If any of the three criteria are not met, regulation (EC) No 853/2004 will apply, and the establishment will have to be approved by the Swedish Food Agency instead (The Swedish Food Agency, 2024a) as a GHE and comply to (EC) No 853/2004 as well.

Thus, registered LRE which handle, and process uninspected wild boar carcasses should apply for approval as GHE, and are, in a sense, “wrongfully” registered as LRE under the current legislation.

The main activity of the LRE must be the direct supply of products to the consumer. The extent to which this is allowed is not further defined. However, to be classified as a LRE complying to strictly (EC) No 852/2004, the main activity of the establishment must be retail oriented. In other words, sales to consumers should be done to such an extent that the establishment would have to be registered as a food establishment, even if only direct deliveries to consumers are taking place. Thus, deliveries to other establishments in Sweden, on the common market or third country outside of EU cannot be the main activity (The Swedish Food Agency, 2024a).

Shops and restaurants, as LRE, can be registered to receive small amounts of game meat, other than for example wild boar or bear, directly from a hunter. If these types of establishments handle game meat products, the FBO has to declare it in their business description and in their registration form at the local municipalities. These types of deliveries do not require any official meat inspection. However, at the moment, wild boar is not included in these regulations and must still be delivered to a GHE for inspection (The Swedish Food Agency, 2023e). Still, and as stated previously, wild boar is processed (unlawfully) in such establishments as well as in unregistered local establishments (Jordbruksverket, 2013; SVT NYHETER/ Halland, 2020).

3.3 Hunter Practices (H)

Hygiene connected to hunting is a concept that includes the method of hunting, shooting, disembowelling, and transport of the carcass as well as the chilling and the cutting of the meat (Paulsen, 2011). Game meat hygiene is affected by a multitude of factors such as the animal’s health before the shot, the attitude and skill of the hunter, the target position, and the behaviour of the animal after shooting (Atanassova *et al.* 2008). Depending on the prevailing conditions, which equipment and premises the hunter has access to, the method and techniques of choice to handle the carcass vary as well. However, it is common to dress the game either laying down and placed on its right side, preferably where the ground is slightly tilted, or alternatively, hanging up. Horizontal and hanging evisceration techniques have different advantages, but whatever the method, the most important thing is that contamination of the body cavity and muscle tissue from faeces and soil is minimised or prevented, and that inspection of the meat is not made difficult (Paulsen, 2011).

Placement of the shot and its effect on hygiene

Hunting hygiene begins from the moment the shot is fired. A correctly placed shot not only inflicts immediate death, which is relevant from an animal welfare perspective, but also facilitates the collection of the game and allows an early evisceration. The mode of killing and the location of the shot also shows a distinct influence on the microbial numbers in deep muscle tissues (Laaksonen & Paulsen, 2015; Paulsen *et al.* 2011; Atanassova *et al.* 2008). For example, a desired target that increases meat quality when it comes to wild boar is the thoracic cavity. The intention is for the blood to cause damage to the lung- or heart tissue, causing exsanguination of the animal as the blood fills the chest cavity leaving other tissues. This makes further bleeding unnecessary and lets the massive blood flow wash away contaminants rather than spreading them (Laaksonen & Paulsen, 2015; Paulsen *et al.* 2011). Abdominal shot lesions on the other hand, are associated with contamination of the exposed muscles and abdominal cavity by introducing microbes from the intestinal tract to other tissue through the circulatory system. As the intestinal tract contains a high concentration of bacteria, their presence in meat often indicates faecal contamination during shooting or slaughter. Unnecessary incisions and bleeding of the carcass risk transferring bacteria from the hide into the chest and exposes tissue for contaminants during transport (Atanassova *et al.* 2008; Paulsen *et al.* 2011; Laaksonen & Paulsen, 2015). Additionally, improper treatment of the shot wounds and unsanitary handling of the game during processing can cause decay by both aerobic and anaerobic bacteria (Atanassova *et al.* 2008).

General hygiene practices

While handling the carcass during the slaughtering process, good hygiene is facilitated by gloves; rolled up sleeves or sleeve covers; clean hands without cuts, rings, and other jewellery; clean equipment; and, refraining from placing the knives on the animal's skin or on the ground. Furthermore, alternating between touching the animal's fur and the body cavity should be avoided due to the risk of transferring contaminants (Laaksonen & Paulsen, 2015). If any intestinal content has leaked out, paper, a knife or a rug is suggested to remove as much of the leakage as possible (Svenska Jägareförbundet, 2017). However, Laaksonen & Paulsen do not agree on the use of paper, a cloth, or other porous materials. Instead, the inside of the abdominal and thoracic cavity can be cleaned with sprayed water as long as the water is not used to rinse the skinned carcass surface. A favourable option advocated by several authors is that impurities, tendons, and gunshot wounds are trimmed away with a good margin to ensure hygienic meat (Gill, 2007; Radakovic & Fletcher, 2011; Laaksonen & Paulsen, 2015).

Hygiene during disembowelling and evisceration

A key aspect when disembowelling is the timeframe where evisceration should be executed without undue delay (Laaksonen & Paulsen 2015; Winkelmayer *et al.* 2008 see Paulsen *et al.* 2011). Winkelmayer *et al.* (2008) proposes a maximum of 3 hours to reduce the risk of microbial spread and prevent non-bacterial spoilage of overly warm carcasses while Laaksonen & Paulsen (2015) advocate a time span of no longer than 1 hour after the kill. Although, if there has been a hit in the digestive tract, evisceration should be done immediately to reduce the risk of spreading bacteria from the intestines (Laaksonen & Paulsen 2015).

Disembowelling is usually executed in two steps. One step is for the hunter to remove the stomach, the spleen, the kidneys, the heart, the lungs, the liver, and the small and large intestines. To avoid blood filling up the abdominal cavity reducing the hunter's oversight over the carcass, it is recommended to start in the abdominal area. The other step is to remove the rectum, urinary bladder, and genitals (Svenska Jägareförbundet, 2017). These steps can be performed out of sequence, and it is common that the digestive tract is removed outdoors. If the hunter chooses to disembowel the wild boar in the forest, the hunter must take extra hygienic precautions as the resources concerning personal hygiene are limited and contamination from the surroundings can occur (Laaksonen & Paulsen 2015). To preserve the tenderloin, kidneys and perirenal fat can be saved for later removal (Casoli *et al.* 2005).

A common procedure when it comes to the removal of organs in the forest is by starting to place the wild boar on its back and holding back the hind legs while making an incision behind the tip of the sternum. It is also preferred to place the game on slightly tilting grounds, as this simplifies removal of the intestines. When the incision is made, it is important to be careful not to puncture any internal organs such as the stomach, urinary bladder, or the intestines that otherwise risks contaminating the meat. The incision is continued until it reaches the abdominal cavity followed by an incision that reaches the pelvis. However, if the cut in the abdominal region becomes too long, this can increase the risk of impurities entering the carcass during transport. When the abdominal cavity is opened, the membranes that attach the digestive tracts are carefully loosened by hand or, if the membranes are tough, using a knife. At this stage, the oesophagus and rectum are sealed off with ties to prevent stomach content or faeces from contaminating the meat. This method protects remaining organs such as the heart, lungs, kidneys, and liver while the carcass is transported to the location of skinning and removal. Lastly, a hole can be cut at the top of the diaphragm to let the blood flow out from the thoracic cavity. It is crucial that the carcass is not turned over to avoid soil and other contaminants from entering the carcass. If there is no delay for transport to skinning, removal of the digestive tract can be done at the same facility as skinning (Laaksonen & Paulsen 2015).

The rectum, urinary bladder and reproductive organs can be removed at various stages. It can be removed in the field or at the slaughter facility. One method described by Laaksonen & Paulsen (2015) is to remove these organs during the stage of skinning, however they do point out that there are other useful methods as well and that the main focus is to avoid recontamination. When removing the urinary bladder, reproductive organs, glands and the rectum, care must be taken so that their contents do not come into contact with the abdominal cavity.

Inspection of the carcass and internal organs is also a part of the evisceration process if the meat is to become food. If no special trained person has examined the carcass, all red organs (heart, liver, lungs, spleen, and kidneys) as well as the head and diaphragm need to accompany the carcass to the GHE. However, if a hunter that has completed game examination courses has inspected the carcass and all eviscerated viscera for signs that may indicate illness and present a health risk, only the head and the diaphragm need to be sent along with the carcass. The trained person also needs fill in a form to declare that all organs were healthy. If any abnormalities are found during the examination, or any abnormal behaviour of the animal has been noticed before the harvest, all deviations must be noted in a deviation form accompanying the carcass and all thoracic and abdominal organs to the GHE, where an inspecting veterinarian will decide whether or not the meat is fit for human consumption (Naturvårdsverket, 2014; EC 853/2004).

The examination shall be carried out as soon as possible after slaughter. Since wild boars are prone to carrying *Trichinella*, the head and all internal organs besides bowels and the stomach must accompany the carcass to a GHE for inspection to ensure that the meat is safe to consume (EC 853/2004; Casoli *et al.* 2005).

Transport

Transporting large game carcasses such as wild boar from the shooting site can be challenging. From a hygiene perspective, dragging the game directly on the ground is a less favourable option as the carcass can catch both soil and other dirt. Instead, Paulsen & Laaksonen (2015) suggest using a plastic sled or plates that have been specially designed for the purpose to transport the carcass. It is also advisable not to stack game carcasses on top of each other to avoid cross contamination.

Hanging conditions

Despite good hygiene practices, some bacteria will always enter the carcass during slaughter, and cold temperatures can help hinder or reduce the growth of pathogenic or spoilage bacteria in the meat (Laaksonen & Paulsen, 2015). The skin can serve as a barrier to protect the meat from bacterial contamination during the hanging period (Svenska Jägareförbundet, 2017). Game meat can be hung and stored at 0-3 ℃ for 3-14 days (sometimes up to 20) if the relative humidity is around 90-95% and provided that the previous hunting hygiene has been adequate. Note the hanging time varies between species, for example, wild boars require less hanging time than ruminants (Laaksonen & Paulsen, 2015).

Hygiene during skinning

To reduce the risk of contaminating the meat, skinning of the carcass must be done with hygiene in mind, whether the game is skinned in the field or in a specialised area. Using clean tools throughout the process is of outmost importance as the carcass may be exposed to faecal, skin or soil bacteria from contact with the used equipment, the skinner's hands or the hide itself (Casoli *et al.* 2005; Laaksonen & Paulsen, 2015). Thus, freshly exposed fat and surface tissues must never be in contact with the outer surface of the carcass. A carcass can be skinned by being strung up at the top of the tendons of the legs that is closest to the hooves, or it can be placed on its back in a stand/cradle. All equipment such as knifes should be cleaned and sterilised in steam or water- heated to at least 82 °C. By cutting the hide from the inside and out, skin and hairs are prevented from getting into the carcass. The head can be detached either before or after skinning (Laaksonen & Paulsen, 2015).

Hygiene during cutting

Using (EC) No 2/2004 as a basis, van Schalkwyk *et al.* (2011) highlighted that the process of cutting and boning must be organised in a way that will prevent or reduce contamination. Meat that is to be cut up should be brought into the workspace progressively and should be maintained at a temperature of not more than 7 °C during processing with an ambient temperature not to exceed 12 °C (van Schalkwyk *et al.* 2011).

In smaller slaughter premises, evisceration and skinning operations may be carried out in the same areas as where the meat is to be cut up. If this is the case, these operations must be performed at separate times. That is, slaughter must not take place at the same time as other processing. Similarly, the premises must be thoroughly sanitised, washed and disinfected before switching between operations. If possible, it is beneficial to have a separate section or room for grinding, slicing or vacuum packing. Otherwise surfaces and machines will be more complicated to keep clean during slaughter and washing (Laaksonen & Paulsen, 2015).

3.4 Conflicting views concerning slaughter hygiene quality

Tradition plays an important role in hunting and thus, in the handling of the killed game. Criticism that certain traditions have negative consequences for meat hygiene has been raised by, among others, Paulsen *et al*. (2011). Used to exemplify the issue is a past tradition which required that shirt sleeves should not be rolled up and that protective gloves should not be worn during evisceration, so-called 'red labour'. However, this practice was abandoned during the 1970s. A still widespread ritual is to present the game after the hunt, which is not without hygienic complications, especially if the outside temperature is more than 12 °C and already eviscerated carcasses are placed on the ground (Paulsen *et al.* 2011).

Other concerns within the topic of the hygiene of hunters have also been raised. It has been debated whether hunters' knowledge concerning food hygiene and meat quality is sufficient to ensure safe products. One concern is the transport of unskinned carcasses and that hunters may have poor attitudes towards hygiene during this step. Another concern is that hunters would let the time frame between the hunt and the slaughter become too long (Naturvårdsverket, 2014). The Swedish Food Agency argued similarly regarding the hunter's level of knowledge of hygiene and food safety, and used skinning, slaughtering and further processing as the most sensitive aspects in the discussion of food hygiene (The Swedish Food Agency, 2021). A study by Wennborg *et al.* (2021) reported the same issues, where several actors involved in the wild game supply-chain voiced their concerns, postulating that hunters' general knowledge of food hygiene, including handling and disposal are substandard. Game dealers also argued that available educations for hunters concerning game handling lacked practical elements (Wennborg 2021). As a reason for this inadequate knowledge, Naturvårdsverket (2014) writes that hunter organisations and authorities have not required hunters to have adequate knowledge of slaughter and meat handling previously, primarily because the main consumers have been the hunters themselves and their families. Thus, there has been no actual market requirement for quality characteristics of game meat from hunters before.

To ensure that a hunter's deliveries of small quantities of wild boar and wild boar meat are compatible with the requirements for a high level of protection of human health, the Swedish Food Agency is of the opinion that a hunter who wishes to supply small quantities of wild boar or wild boar meat directly to end consumers must undergo special education. The suggested education would be covered over two courses (The Swedish Food Agency, 2021). The first one is a game inspector course as set out in Chapter I of Section IV of Annex III to Regulation (EC) No 853/2004: this allows hunters to inspect game before it is delivered to a GHE and is usually organised by hunter organisations. However, the curriculum is audited and approved by the Swedish Food Agency (The Swedish Food Agency, 2021). The second course would instead be specialised around hygienic handling, sampling, and traceability of slaughter processes for wild boar and wild boar meat (The Swedish Food Agency, 2021). Such wild boar courses have not yet been approved by the Swedish Food Agency as of May 2024, although, there is an ongoing process. Besides schooling in hygiene and examination of wild game, the hunter would also need to register at the county board to receive a personal code for tracing products. The trace code is an important part of the supply chain when the meat is to be sold or donated (The Swedish Food Agency, 2024c).

Naturvårdsverket (2014) and The Swedish Food Agency (2021) did not raise similar concerns regarding game carcasses submitted to GHE, because carcasses and organs are inspected by an inspecting veterinarian and treated by professional staff who are responsible for quality (Naturvårdsverket, 2014; The Swedish Food Agency, 2021). Also, a GHE most often has better facilities for game handling from the start compared to a hunter's own premises (The Swedish Food Agency, 2021).

In Wennborg's study (2021), however, the opposing opinion of other actors postulating hunter's knowledge was also presented. This view is also supported by Gill (2007) and Atanassova *et al.* (2008) who state that the hygienic quality of game meat handled correctly at all stages of processing such as slaughter, and cooling in a suitable manner, whether it has been handled at a GHE or at an equivalent facility of the hunters, can be equally good, or better, than meat from domestic animals slaughtered in approved slaughterhouses (Gill, 2007; Atanassova *et al.* 2008).

A study from Serbia points in the same direction, that hygiene is not always better in a GHE compared to in the field, and that the way the carcass is handled and eviscerated plays a significant role (Mirceta *et al.* 2017). The same study also highlighted that few of those who handled the carcasses at the facilities had training. Gomes-Neves *et al. (*2021) summarized studies from 2010 and 2020 from across Europe. The variation in the number of bacteria and which species were detected on the carcasses turned out to be quite high. This may, in addition to different sampling methods, depend on how the game has been handled in the past, and the different environments and climates it has been exposed to. The latter also applied to other game species that were examined (Gomes-Neves *et al.* 2021).

Besides slaughter hygiene, the results of the Serbian study also indicated that wild boar had the greatest variation when it came to bacterial presence compared to other game species and had the largest proportion of unapproved carcasses (Gomes-Neves *et al.* 2021). Similar results were reported in an Italian study where relatively high contamination of various bacteria was seen on wild boar in general. However, there was some variation in the amount and type of bacteria found on carcasses slaughtered in private households and registered establishments (Peruzy *et al.* 2022), and the number of colony-forming bacteria (CFU) was always below the limit value set by the EU of <5 log CFU/cm^2 for domestic pigs ((EC) No 1441/2007).

To further strengthen the knowledge about the importance of hygiene, or to increase knowledge in safe game meat handling in general, Paulsen *et al.* (2011) and Gomes-Neves *et al.* (2021) both highlight cooperation and compliance of hunters as a key element to improve game meat hygiene and suggest that more concentrated efforts should be made (Paulsen *et al.* 2011; Gomes-Neves *et al.* 2021). An Austrian example where cooperation with the hunter community has proved successful describes that a more organised handling, slaughter, and chilling of wild game together with pre- and post-slaughter inspection of carcasses resulted in an increase of carcasses that met hygienic requirements (Paulsen, *et al.*, 2011). The hunters' attitudes towards an increased learning of pathology, food safety and slaughter hygiene were studied by Wennborg (2021). There, results showed that 90% of the responding hunters claimed that they were interested in further training in pathology and food hygiene.

3.5 Hygiene Criteria and Microbes

It is clear that slaughter and further processing of wild boar meat is an important part of ensuring food hygiene, otherwise, poor hygiene risks causing food poisoning for the final consumer (Gomes-Neves *et al.* 2021, Mirceta *et al.* 2017). Paulsen *et al.* conclude that there is a risk for bacterial and fungal contamination of the meat surface throughout all subsequent stages of the production cycle. Depending on the water activity of the meat and the surrounding temperature, bacterial multiplication will vary (Paulsen *et al.* 2011).

Common bacteria that can cause food poisoning or spoilage found in wild boar meat include, *Escherichia coli, Campylobacter, Yersinia enterocolitica, Yersinia pseudotuberculosis, Listeria* spp.*, Clostridium, Salmonella* spp.*, Klebsiella* and *Citrobacter* (Gomes-Neves *et al.* 2021; The Swedish Food Agency, 2019b; Atanassova *et al.* 2007; Da Silva *et al.*, 2018). It has been reported that both *Y. enterocolitica* and *L. monocytes* have been recovered from store bought wild boar meat and from faecal contaminations found on wild boar carcasses, although not all strains that were identified were pathogenic (Gill, 2007). There are also many reports of *Listeria* occurring on wild boar carcasses. Although, something that needs to be taken under consideration when discussing *Listeria,* is that it frequently occurs also in later production stages such as game chambers, cold rooms, and processing equipment at slaughtering facilities (Gill, 2007).

Since the microbiological quality of meat often determines its shelf life, measuring the total number of bacteria growing on the meat is a common hygiene indicator (Naturvårdsverket, 2014). If there has been unsanitary slaughtering or handling conditions, a slow cooling process of the carcass, or an incorrect storage temperature, these bacterial levels can rise. However, the total amount of mesophilic aerobic bacteria only serves as indication; the method does not identify any genera of pathogenic bacteria and is therefore not a direct measure of the risk for foodborne disease. On the contrary, it can sometimes be the case that a natural non-specific microflora can inhibit the growth of pathogenic microorganisms (The Swedish Food Agency, 2023f; Giacometti Cavalheiro *et al.* 2022). Moreover, members from the *Enterobacteriaceae* family have also been recovered in chilled environments of slaughtering facilities and can be used as an indicator for contamination of enteric bacteria such as Shiga toxin producing strains of *E. coli* (EHEC, responsible for symptoms such as bloody diarrhoea and kidney failures), *Yersinia* spp. and S*almonella enterica* (Terezinha Souza Macedo & Teresinha Van Der Sand, 2005; Giacometti Cavalheiro *et al.* 2022; Da Silva *et al.*, 2018; The Public Health Agency of Sweden, 2020). Several *Enterobacteriaceae* are pathogenic to humans, posing a serious health hazard. *Enterobacteriaceae* is also able to colonise niches in processing plants where sanitation and cleaning are inadequate (Da Silva *et al.*, 2018).

Other factors that can inflict on the microbial load is the meats pH value and cooling temperature (Naturvårdsverket, 2014). In a study made by Samples *et al.* (2023) wild boar meat proved a higher pH compared to domestic pigs and varied from 5.55 to 5.87 while the domestic pigs had a pH around 5.48-5.52. The wild boar meats' higher pH is a probable result of higher activity before slaughter, thus resulting in lower amount of available glycogen. This in turn can lead to a shortened shelf life as the risk for microbial growth increases (Samples *et al.* 2023).

To evaluate the hygienic status of game meat, a mesophilic aerobic bacteria count, the presence of possible pathogens, and the number of *Enterobacteriaceae* can be used (Atanassova *et al.* 2007). However, there are as yet no specific microbiological criteria for wild boar carcasses or wild boar meat within the European Union. There are, however, other regulations applying to the microbial criteria for pork carcasses found in Regulation (EC) No 2073/2005, or a limit value of 7 $\log_{10}$ cfu/g set for aerobic bacteria in meat which can be used as hygiene indicators on a voluntarily basis (Peruzy *et al.* 2022; Naturvårdsverket, 2014). The (EC) No 2073/2005 regulation has also set limits for minced meat, which is one of the most common wild boar products that was bought by public sector organisations in 2020, according to a national survey by MATtanken (2021) commissioned by Jordbruksverket.

The microbes in minced meat that are regulated by (EC) No 2073/2005 are aerobic mesophilic bacteria and *E. coli*, and their limit values are stated in Table 1. In Table 1, there are also suggested actions how to reduce exceeding bacteria levels from the regulation (EC) 2073/2005. These hygienic criteria are to be applied at the end of the manufacturing process and when subsampling has taken place. To describe the overall hygiene standard of a minced meat sample, three hygiene classifications have been made using m and M as a lower and upper limit, *c* for the number of sample units giving values between m and M, and *n* for the number of units comprising the sample. Firstly, there is the classification '*satisfactory'* which means that all the observed values are less than the m limit. The second classification, '*acceptable*', is generated if a maximum of c/n values are between m and M, and the rest of the values are below m. Lastly, there is '*unsatisfactory'.* A sample will be classified as such, if one or more of the values observed are >M or more than c/n values are between m and M. (EC No 2073/2005).

Table 1. Process hygiene criterions for minced meat applied at the end of the manufacturing process (Regulation (EC) No 2073/2005).

Microorganisms	**Limits**		**Limits in log** [c]		**Actions in case of unsatisfactory results**
	m	**M**	**m**	**M**	
Aerobic colonies [a]	$5x10^5$ cfu/g	$5x10^6$ cfu/g	5.7 log cfu/g	6.7 log cfu/g	Improvements in production hygiene and in selection and/or origin of raw material
E. coli [b]	50 cfu/g	500 cfu/g	1.7 log cfu/g	2.7 log cfu/g	Improvements in production hygiene and in selection and/or origin of raw material

[a] The criterion is not applicable on retail levelled produced minced meat when the self-life is less than 24 hours.

[b] Used as an indicator of faecal contamination.

[c] To facilitate comparison of sample results to compliance with hygienic criteria, the criteria were translated into log cfu/g.

To perform an analysis of these bacteria in accordance with the regulations, ISO certified methods are to be used (EC 2073/2005). Counts of *Enterobacteriaceae* can be used as general indicators of hygiene, although specific limits for this group are not available for minced meat. Further identification of *Enterobacteriaceae* colonies using Maldi-Tof (Peruzy *et al.* 2019) can help provide hints regarding origin of the bacteria and if they are normally found in the soil, faeces or have some other common source.

4. Material and Methods

The section below will present the contact and sampling procedures, sample preparation and plate count methods that have been used. Good laboratory practice was applied throughout.

4.1 Contacts for sample collection

Material was collected for analysis from hunters, GHE and LRE around the middle and southern parts of Sweden. All participants that contributed with samples were informed on what type of analysis that was being made. Contributions to the study were done on a voluntary basis. Appointments for sample collection were booked via phone calls, emails or text messages and information about the samples were mainly gathered upon collection of the samples through conversation. If additional questions were needed, phone calls, text messages or emails were used to collate the required information.

4.2 Sampling protocol

Upon collection of H and LRE samples, a protocol was filled in to gather information concerning the samples. The full sample protocol is included in Appendix 1.

The protocol included details such as the number of samples, whether the samples consisted of cuts from one or several carcasses, where the dressing and evisceration of the carcass had taken place, at which temperature the sample had been hung for tenderisation, and date for mincing. The protocol was also used to collect information on how the game was hung, and the temperature at which the meat was cut and minced. The 'other information' section in the form, collected other available information such as how the shot hit the wild boar, if the animal's rectum, urinary bladder, and genitals were removed in the forest or at a facility, and how the hunt was conducted such as if a dog had been used during the hunt to drive the animal or if the animal was stalked. These sections were filled in with the co-operation of the sample suppliers. Details such as the sample temperature upon collection and average temperature at arrival to the laboratory and pH of the mince were also noted.

4.3 Sampling and storage conditions

Sampling took place at the end of January to May of 2024. The carcasses had been hung between 3 to 25 days in temperatures ranging from 2-10 °C. All samples were freshly minced and packaged in freezer bags or vacuum sealed bags during transport.

All H and LRE samples had their approximate temperature measured with a cooking thermometer when collected in order to gain an overview of the storage temperature. To avoid contamination of the samples, the temperature was measured from outside of the bag by folding the minced meat around the thermometer. The protocol was filled in together with the sample provider.

The H and LRE samples were transported in a cooler bag and driven directly to the laboratory for testing to avoid an unbroken cold chain. Before measuring the temperature and pH, all samples were mixed by hand through the bag in order to even out the bacterial load, pH, and temperature through the sample. Each parameter was measured at 5 different places to gain a fuller picture of the sample and a mean value was calculated from it. The temperature measurement took place first, and the thermometer was washed and sanitised with 70% ethanol before use. The pH-meter (Knick - Portamess 913) was rinsed with deionised water and sanitised before being used and rinsed between every measuring point. Furthermore, the pH-meter was calibrated weekly using technical buffer pH 7.00 and 4.01 (Mettler Toledo). For each sample, the temperature settings of the pH meter were adapted to the sample's average temperature.

Samples from the GHE were delivered by an external company and were transported at chilled conditions ranging from + 2-8 °C. All GHE samples were produced the same day but were collected at three different times during the production day, 8:00, 11:30 and 14:00. Each GHE sample had its collection time noted on the bag. The samples were delivered in vacuum sealed bags and their temperature and pH were measured using the previously described method.

The microbial analysis was initiated after pH and temperature measurements. No further blending of the sample was deemed necessary as all had been mixed thoroughly before the temperature and pH measurements. For the majority of samples, microbial analysis took place within 24 hours of mincing: for certain samples, for logistical reasons, microbial analysis was performed up to 4 days after mincing, during which time the samples were stored between 2-8 °C.

4.4 Enumeration of microbes

4.4.1 Sample preparation

Sterile peptone water (SPW) diluent was prepared according to ISO 6887-1:2017 and ISO 6687-5:2012 and consisted of 1 g of enzymatically digested casein, 8.5 g of sodium chloride and 1 L of distilled water that was autoclaved for 15 minutes at 121°C (Da Silva *et al.*, 2018). Each sample of minced meat was flattened through the plastic bag to a 2-3 cm thick disk. A sub-sample of 25 g was taken from the middle of the disk and was transferred into a sterile stomacher bag with 225 ml of SPW followed by homogenisation in a Stomacher 400 (Seward) at normal speed for 60 seconds. Serial dilutions were made in SPW up to 10^{-7}.

4.4.2 Plate count of *Enterobacteriaceae*

The plate count procedure was carried out according to ISO 21528-2:2017. Aliquots (1 mL) of the serial dilutions were transferred to a petri dish and mixed with Violet Red Bile Agar (VRBG; Oxid CM0485) that had been brought to 46 °C. The inoculum was mixed through circular movements, 8–10 clockwise and 8–10 times counterclockwise.

After solidification, an additional layer of media of 5–10 ml was added to the plates. The plates were incubated at 37 °C for 24 ± 2 hours.

Plates with less than 150 colonies were chosen for counting, and a typical colony had a dark purple colour (Da Silva *et al.*, 2018).

Identification and differentiation

In order to identify and differentiate between colonies found on the VRBG plates, 5 colonies in total were randomly chosen for each sample. The colonies were purified onto general media (brain heart infusion agar or nutrient agar) and oxidase tested to confirm that oxidase negative colonies had been chosen, as *Enterobacteriaceae* are oxidase negative (Da Silva *et al.*, 2018). Colonies that were around 24 hours old were identified by MALDI-TOF analysis using a Bruker – Microflex LT.

4.4.3 Plate count of *E. coli*

Based on hygienic criterions for minced meat regulated in (EC) NO 2073/2005, *E. coli* was quantified according to ISO 16649 – 2:2021. Chromocult tryptone bile X-glucuronide agar (TBX; Millipore) was autoclaved and brought to 46 °C before plates were poured.

Aliquots (1 mL) of serial dilutions were transferred to petri dishes in duplicate, and TBX added as described above. The time between distribution of the inoculum and pouring did not exceed 15 minutes. In order to allow resuscitation of stressed cells, the plates were incubated for an initial period of 4 hours in 37 °C, before incubation at 44 °C for 20–24 hours. All blue and blue-green colonies that could be detected on the plates were counted (Merck, 2019; International Standardisation Organisation, 2021).

4.4.4 Plate count of Aerobic mesophilic bacteria

Total aerobic mesophilic bacteria plate count was made in accordance with the ISO 4833-1:2013 method in order to examine the levels of bacteria with the regulated levels stated in (EC) NO 2073/2005. The plate count agar (PCA) contained 5 g of tryptone, 2.5 g of yeast extract, 1 g dextrose, 15 g of agar and 1 L of distilled water. It was sterilised under standard conditions and tempered to 46 °C before 1 ml of the sample dilution was pipetted into a petri dish and mixed with the PCA. All plates were allowed to solidify on a flat surface before being incubated at 30 °C for 72 ± 3 hours. Plates with around 30-300 colonies were counted (Da Silva *et al.*, 2018).

4.4.5 Calculation of colony forming units per gram

Calculations of cfu/gram were performed according to the general formula (Da Silva *et al.*, 2018):

$$\text{Cfu/g} = \sum C \,/\, v \text{ x } [n_1 + (0.1 \times n_2)] \times d$$

Where:

$\sum C$ = the sum of typical colonies counted on the plates selected for counting from two successive dilutions.
v = the volume that is inoculated on each plate (1 mL).
n_1 = number of plates counted from the first dilution
n_2= number of plates counted from the second dilution
d = the first dilution retained from counting

Neither the total aerobic bacteria nor *Enterobacteriaceae* plates were prepared as replicates, thus one plate per dilution was counted. However, the *E. coli* plates were prepared with duplicate replicates per dilution and the mean value of the duplicates was used in the formula.

4.5 Statistical analysis

To compare aerobic bacteria, *E. coli* and *Enterobacteriaceae* counts between the three sample categories of hunter, LRE and GHE samples, an analysis of variance (ANOVA) was calculated, using the software R. Statistical raw data were processed in Excel. To detect differences between the bacterial groups, the p-value of significant results were assessed with a Tukey's Honest Significant Difference test (*Post hoc*- test) with a probability value of 0.05; p-values less than 0.05 ($p < 0.05$) was defined as statistically significant. In order to obtain a normal distribution, the logarithmical values of the bacterial counts were used. A non-parametric test, which did not require a normal distribution, was also performed to verify the parametric test. The results were equivalent, and the non-parametric test was therefore not included in the report.

5. Results

The average microbiological load for each sample category is given here, as well as the standard deviations, identified bacterial species, average hanging period and hygienic quality. Individual sample data are available in Appendices 2 and 3. To assess the hygienic quality of the samples, the hygienic processing criteria for minced meat in (EC) No 2073/2005 have been used. Note that the classification terms *satisfactory, acceptable,* and *unsatisfactory* are not fully applicable, as no sub-sampling has taken place as stated in the legislation. However, the criteria have served as a basis to determine the hygienic quality of the samples.

5.1 Microbial counts

As seen in Table 2, a total of 39 samples were analysed, of which 29 would be classified as "*unsatisfactory*" when compared to the hygiene criteria for minced meat given in (EC) No 2073/2005. The majority of the samples that were rated as *unsatisfactory* were found in the GHE category. Of the remaining samples, 6 were classified as *acceptable* and 4 as *satisfactory* according to the hygienic criteria limits (Table 2). The largest proportion of unsatisfactory samples belonged to the GHE category with 17 out of 17 samples (100%), followed by the LRE category with 8 out of 11 (73 %) and lastly, the H samples with 4 out of 11 (36 %) unsatisfactory samples (Table 2).

Data concerning average hanging period of a carcass is also presented in Table 2. The average period for a H carcass to be hung in this study was 7 days while carcasses collected from LREs had the longest hanging period of 16 days as an average. GHE carcasses had the shortest period for hanging carcasses, with an average of 3-4 days (Table 2).

Table 2. Number and percentage of samples for each category – hunters (H), local retail facilities (LRE) and game handling facilities (GHE) – that were classified as approved, acceptable, or unsatisfactory based on the hygienic criterions of (EC) No 2073/2005. Calculated from individual sample data available in Table 5, Appendix 2.

Sample	Number of samples	Satisfactory	Acceptable	Unsatisfactory	Average hanging period (days)
H	11	3 (27.2 %)	4 (36.4 %)	4 (36.4 %)	7
LRE[b]	11	1 (9.1 %)	2 (18.2 %)	8 (72.7 %)	16
GHE	17	-[a]	-[a]	17 (100 %)	3-4

[a] No samples were within the hygienic criteria for the given description.

[b] The table uses the term Local retail establishments as defined in (EC) No 853/2004 to describe establishments that has been wrongfully registered for wild boar meat handling, or establishments with similar traits of an actual local retail establishment but handles wild boar meat unregistered (illegal).

Table 3 shows the average total mesophilic aerobic bacteria for H, LRE and GHE samples, namely, 6.1 log cfu/g, 7.3 log cfu/g and 7.7 log cfu/g respectively. GHEs had a slightly higher average, 7.7 log cu/g, compared to LREs (7.3 log cfu/g). The average value for aerobic bacteria in H samples (6.1 log cfu/g) met the hygienic criteria upper limit of EC No 2073/2005 of 6.7 log cfu/g. This average value for H samples was significantly lower than the average value for LRE and GHE samples, according to the statistical analysis presented later in section 5.1.

Regarding *E. coli*, the LRE samples had the highest average levels (2.4 log cfu/g) followed by the GHE and H samples (2.1 log cfu/g), shown in Table 3. As for the average *Enterobacteriaceae* amounts, the H samples contained 4.0 log cfu/g whereas the LRE samples showed 3.8 log cfu/g and the GHE samples, 4.5 log cfu/g (Table 3).

In total, 12 different genera from the *Enterobacteriaceae* family were identified in the samples, namely: *Hafnia, Escherichia, Yersinia, Serratia, Leclercia, Klebsiella, Enterobacter, Buttiauxella, Pseudescherichia, Pantoea, Citrobacter* and *Kalamiella* (Table 3). Four genera were represented by two species, namely *Serratia* spp., *Yersinia* spp., *Pantoea* spp. and *Klebsiella* spp. were detected. The samples that had the largest variation in the bacterial flora, in terms of number of species, were the LRE samples. Amongst the LRE, 11 species were identified whereas 7 species were found in the GHE samples. The hunter samples had a variation of 10 species in total.

Out of the bacteria, *Hafnia alvei, E. coli, Buttiauxella gaviniae,* and *Serratia fonticola* were recovered in all sample groups. Bacterial species only found in two out of the three groups were: *Serratia liquefaciens, Klebisella oxytoca* and *Leclercia adecarboxylata* in LRE and H samples; and *Yersinia enterocolitica* in both H and GHE samples. Both species of *Pantoea septica* were only recovered from H samples. The LRE samples also had bacterial species specific to the sample group: *Pseudescherichia vulneris, Kalamiella piersonii, Citrobacter gilleni* and *Enterobacter bugandensis*. Likewise, *Yersinia kristensenii* and *Klebsiella pneumoniae* were only reported from GHE samples (Table 3).

Table 3. Average bacterial counts (log cfu/g ± std deviation) in hunter (H), local retail establishment (LRE) and game handling establishment (GHE) samples. Identified Enterobacteriaceae from all samples are listed (Individual sample data can be seen in Table 5, Appendix 3).

Samples	**Aerobic**	***E. coli*[a]**	***Enterobacteriaceae*[a]**	**Identified *Enterobacteriaceae* in order of prevalence**
H	6.1 ± 0.9[b]	2.1 ± 2.3	4.0 ± 2.5	*Hafnia alvei, Pantoea agglomerans, Escherichia coli, Serratia liquefaciens, Serratia fonticola, Leclercia adecarboxylata, Klebsiella oxytoca, Pantoea septica, Buttiauxella gaviniae Yersinia enterocolitica*
LRE[d]	7.3 ± 1.1[c]	2.4 ± 0.9	3.8 ± 1.3	*Escherichia coli, Serratia liquefaciens, Serratia fonticola, Hafnia alvei, Klebsiella oxytoca, Leclercia adecarboxylata, Pseudescherichia vulneris, Kalamiella piersonii, Citrobacter gilleni, Enterobacter bugandensis, Buttiauxella gaviniae*
GHE	7.7 ± 0.3[c]	2.1 ± 0.5	4.5 ± 0.4	*Hafnia alvei, Yersinia kristensenii, Yersinia enterocolitica, Escherichia coli, Serratia fonticola, Buttiauxella gaviniae, Klebsiella pneumoniae*
Significant difference (p-value)	0.00003	0.84	0.46	

[a] For statistical purposes, levels below <5 log cfu/g has been calculated as 2.5 log cfu/g and levels below <10 log cfu/g, was calculated as 5 log cfu/g.

[b, c] Significantly different values according to Tukey's test.

[d] The table uses the term Local retail establishments as defined in (EC) No 853/2004 to describe establishments that has been wrongfully registered for wild boar meat handling, or establishments with similar traits of an actual local retail establishment but handles wild boar meat unregistered (illegal).

5.2 Statistical analysis

The boxplot in Figure 1 shows the degree of dispersion for the bacterial counts of aerobic bacteria, *Enterobacteriaceae* and *E. coli* among the H, LRE and GHE samples.

Firstly, the aerobic bacterial levels of the LRE samples showed the highest spread in log cfu/ g while the GHE had the lowest spread (Figure 1). For the *E. coli* measurements, the H samples showed a substantial variation in the distribution of bacterial levels compared to the LRE and GHE samples. The same pattern can also be seen for the *Enterobacteriaceae* levels, with H samples being the most widely distributed category and GHE being the one with least variation. The greatest variation in bacterial levels for all sample groups were found among the *Enterobacteriaceae* (Figure 1).

The two red lines the in aerobic and *E. coli* boxplots illustrates the (EC) No 2073/2005 hygiene criteria span. It can be seen that most of the sample dispersion of H samples is found within the hygienic span for aerobic bacteria, whilst *E. coli* levels of H samples are more scattered.

In relation to the hygiene criteria, the GHE log cfu/g values for total aerobic bacteria are seen well above the upper limit. The LRE samples bacterial counts are also widely dispersed above the upper limit. Results for the *E. coli* samples show that GHE are dispersed within the regulated limits and that the LRE samples are spread between middle levels to exceeding the criteria. Figure 1 also demonstrates that H samples are the only sample group with *E. coli* amounts ranging from below the lowest limit up till above the upper limit.

Due to insignificant differences between the samples for *E. coli* and *Enterobacteriaceae* between the sample groups, no Tukey-test was performed for these groups. However, the aerobic bacteria counts showed a significant difference between the samples: the Tukey-test indicated that H samples were significantly different to LRE and GHE, which did not differ from each other (Figure 1).

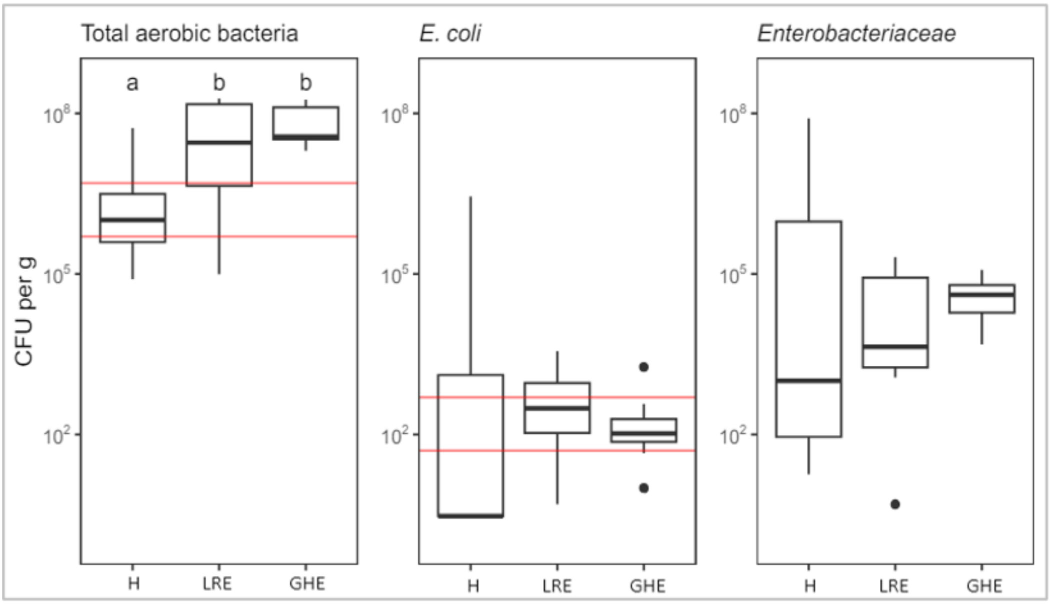

Figure 1. Summary statistics of microbial counts for aerobic bacteria, *E. coli* and *Enterobacteriaceae* in hunter (H), local retail establishment (LRE) and game handling establishment (GHE) samples and the significant difference between the aerobic sample groups, a and b. The height of the squares indicates the overall variation in bacterial levels. Boxes extend to first and third quartiles, with lines indicating the median. Whiskers extend to maximum and minimum values with 1.5 * interquartile range. Outliers are indicated by filled circles. The red lines indicate the hygienic span for aerobic and *E. coli* bacteria to visualise the spread of unsatisfactory or acceptable/satisfactory samples.

6. Discussion

In accordance with the legislative regulations of (EC) No 2073/2005, 29 out of 39 samples in this study would have been classified as '*unsatisfactory*' when compared to the hygiene criteria. Six samples would have been classified as *acceptable* and 4 samples as *satisfactory*. Out of the *unsatisfactory* samples, 17 out of 17 GHE samples, 8 out of 11 LRE samples and 4 out of 11 hunter samples exceeded the hygienic limits. A Serbian study comparing wild boar carcasses to other game species concluded that wild boar had the largest proportions of unapproved carcasses. Even though the study measured bacterial levels in cfu/ cm^2, it can serve as an approximation to support the results of this paper with 29 of 39 unapproved samples.

The fact that there were significantly lower aerobic bacterial counts in H samples than in LRE+GHE samples contradicts the perceived knowledge gap suggested by Naturvårdsverket (2014), The Swedish Food Agency (2021) and Wennborg (2021) concerning slaughter hygiene of hunters compared to an established company. A comparison between the average aerobic counts for LRE and GHE, 7.7 log cfu/g, with the average of 6.7 log cfu/g in H samples, suggests that hunter samples can be produced in a safe manner. These findings are also in line with Mirceta *et al.* (2017) who documented that hygiene is not necessarily better at a GHE than in the field, and that the evisceration and other handling of the carcass has the most significant role rather than the location itself. However, good hygiene is facilitated by having access to adapted premises and proper equipment (The Swedish Food Agency, 2021).

This study is, to the author's knowledge, one of the first which compares bacterial hygienic quality of wild boar meat handled by hunters, unregistered/wrongfully registered local retail establishments, and game handling establishments. The study of Paulsen (2011) compiled several reports using different methods that examined different steps of the supply chain of wild game meat and demonstrated a wide variation in results. Even so, average counts for total aerobic bacteria, *Enterobacteriaceae* and *E. coli* were found to be 3-4, 2-3 and 1-2 log cfu/g, respectively, in those reports. All those values were proposed to be a result of good hygiene practices. The findings of the current study, namely, average levels of 6.7, 7.3 and 7.7 log cfu/g for aerobic counts, 3.8, 4.0 and 4.5 log cfu/g for *Enterobacteriaceae* and 2.1-2.4 log cfu/g for *E. coli*, suggest that hygienic improvement is needed throughout the supply chain to achieve similar results. Paulsen (2011) reported a suggestion for microbial limits of wild game carcasses by Lagrange & Schmidt (2005) set to 5.0 log cfu/g for aerobic bacteria, 3.0 log cfu/g for *Enterobacteriaceae* and 2.0 log cfu/g for *E. coli*. However, these suggestions were made for cuts and not for mince and are lower than those suggested for mince set in the regulation of EC No 2073/2005 (6.7 log cfu/g for aerobic bacteria and 2.7 log cfu/g for *E. coli*). For comparison, expected levels of aerobic bacteria in ground beef are within a range 4-7 log cfu/g (Da Silva *et al.*; 2018), which is more similar to the one set in the EC regulation and the findings of this report.

To reduce the total aerobic count, the Swedish Food Agency (The Swedish Food Agency, 2023f) and (EC) Regulation No 2073/2005, suggests a production review, and to oversee personal hygiene and cleaning practices. As seen in Figure 1, the aerobic count with the GHE group were quite similar, ranging from 7.3-8.3 log cfu/g (Figure 1). The rather even bacterial counts of the GHE samples (Figure 1) could also suggest cross-contamination between carcasses. As slaughtering is carried out on a much larger scale at a slaughtering plant than at a hunter's home, the equipment, such as the mill is not cleaned after each carcass. Thus, one carcass with elevated bacterial numbers is sufficient to cross-contaminate the subsequent carcasses. However, other information concerning previous handling of the GHE carcasses such as placement of the shot, if the animal had been eviscerated in the field etcetera was lacking. Therefore, it is hard to speculate regarding the state of the carcass upon delivery to the GHE. In particular, improper treatment of the shot wounds and unsanitary handling of the game during processing can cause decay by both aerobic and anaerobic bacteria as described by Atanassova *et al.* (2008). If there had not been enough trimming margins regarding the removal of impurities such as tendons or enough trimming around the gunshot wounds, this could also affect the hygienic quality of the meat (Gill, 2007; Radakovic & Fletcher, 2011; Laaksonen & Paulsen, 2015) such as giving rise to increased aerobic levels or *Enterobacteriaceae* counts.

Another reason for higher aerobic values could be due to the timeline between sampling and analysis. Due to logistical issues, the GHE mince was analysed 4 days after mincing, in contrast to the LRE and H samples that were analysed within 24 hours after mincing. A report that addresses this issue is Cenci-Goga *et al.* (2021), who concluded that sampling for an analysis should not be delayed, since proliferation of microorganisms during refrigeration conditions can occur and that chilling may act as a confounder. Furthermore, a higher total aerobic count does not specify the nature of the microbes, if they are pathogenic or not. Thus, some organisms might even inhibit the growth of pathogens (The Swedish Food Agency, 2023f; Giacometti Cavalheiro *et al.* 2022).

Looking into individual samples, when comparing their hanging period to their hygienic grade (Table 4, Appendix 3), a pattern appears among the LRE and H samples. All satisfactory samples were hung for a period of 7 days or shorter, and the acceptable samples had been hung between 3-9 days. Furthermore, samples with a hanging period of 9 days or more were deemed as unsatisfactory. Despite that all GHE samples were reported to have an average hanging period of 3-4 days, their bacterial loads still exceeded the hygiene limits (Table 3). As previously mentioned, details concerning earlier conditions for the GHE carcasses was lacking, complicating these results as carcasses could have been stored for a longer period before being delivered to a collection point or a GHE. As pointed out by Laaksonen & Paulsen (2015), game meat can be hung and stored for 3-14 days (sometimes up to 20) if the storage conditions in terms of humidity and temperature are proper. A temperature between 0-3 °C was recommended. Also, the previous hunting hygiene must be adequate to allow a longer hanging period (Laaksonen & Paulsen, 2015).

When studying the levels of *E. coli* in Table 3, the lowest average count was found in GHE and H samples which both had a corresponding average of 2.1 log cfu/g, whereas LRE samples had the highest average of 2.4 log cfu/g. Compared to the hygienic criteria in EC No 2073/2005, 1.7-2.7 log cfu/g (Table 3), all sample categories had an average bacteria level within the span of the limits. However, the average levels are closer to the upper limit than the lower. According to Terezinha Souza Macedo & Teresinha Van Der Sand, (2005), Giacometti Cavalheiro *et al.* (2022) and Da Silva *et al.* (2018), these results, combined with the average *Enterobacteriaceae* levels (H= 4.0, LRE= 3.8 and GHE= 4.5 log cfu/g), indicate that either faecal contamination has occurred or that improved cleaning practices are needed to reduce these bacterial levels. The p-value for the *E. coli* category was 0.840 (Table 3) which shows that there is no significant difference between the bacterial counts of *E. coli* for the H, LRE or GHE samples. *Enterobacteriaceae* had higher average bacterial counts than *E. coli,* which is to be expected as it includes other member of the *Enterobacteriaceae* family which were identified in the study.

Enterobacteriaceae and *E. coli* contamination may have occurred at several different stages of the game supply chain. As presented in section 3.3, soil or faeces can enter the carcass during evisceration as described by Paulsen (2011), during transport, or by a misplaced shot in the abdomen as gastric and intestinal fluids could have contaminated the carcasses. Abdominal shot lesions can also induce faecal contaminants to the muscles through the circulatory system. Findings in meat usually indicate faecal contamination during shooting or slaughter (Atanassova et al. 2008; Paulsen, 2011; Laaksonen & Paulsen, 2015). Other information collected together with the samples supports this, for example, certain H and LRE samples had damages to their abdominal area through misplaced shot or were hit by a car before being stalked and killed (Appendix 2, Table 4). The samples with levels above the highest *E. coli* limits, such as H4, H5 and LRF 3-6, had counts ranging from 3.3 – 4.1 log cfu/g; this could be explained by Laaksonen & Paulsen (2015) who suggest that evisceration must be done carefully and that the time span of the evisceration is important for such animals, as it otherwise allows the spread of intestinal bacteria. However, the authors also comment that some bacteria will enter the carcass during slaughter despite careful procedures, and that chilling of the carcass can reduce or hinder the microbial growth.

The most frequently identified bacterial species present in all GHE samples, 8 out of 11 H samples and in 2 out the 11 LRE samples, was *Hafnia alvei*. This is a bacteria common in meat products such as mince and which thrives at lower temperatures, such as those of a slaughter establishment or a cold room. Neither is it hindered by vacuum packaging (Ridell, 1999), which would help explain its high prevalence in the samples (Table 4, Appendix 3). *Serratia, Klebsiella* spp., *Citrobacter* and *Pantoea* were also detected in the study. All are able to grow at lower temperatures, similar to those of a slaughtering plant, suggesting that if cleaning is not done properly, they will still be able to colonise (Da Silva *et al.* 2018). As reported by Peruzy *et al.* (2022), some variation in type of bacteria found on carcasses slaughtered in private households and registered establishments were detected. For example, *Pantoea* species were only recovered from H samples, whilst *P. vulneris, K. piersonii, C. gilleni and E. bugandensis* were only recovered from LRE samples. *S. liquefaciens, K. oxytoca* and *L. adecarboxylata* were found in both LRE and H samples and *K. pneumoniae* were recovered from GHE and LRE samples (Table 3.). Of the identified bacteria, the vast majority belonged to the category of food spoilage bacteria or opportunists. Besides *H. alvei* and *Serratia* spp., the latter being able to produce biofilms, there were also findings of *Klebsiella* and *Citrobacter*. These bacteria can for example be found in human faeces, contaminated water, soil and foods (Baylis, 2006), making the time and source of contamination hard to pinpoint. However, a German study regarding *Enterobacteriaceae* findings in meat traced *Citrobacter* and *Klebsiella* findings to the butchers' hands (Mladenovic *et al.* 2021), which suggest improvement in hand hygiene could help reduce their incidence in this study.

In addition, *Y. enterocolitica* was found in the H and GHE samples, whereas *Y. kristensenii* were recovered from GHE and LRE (Table 3). According to Gomes-Neves *et al.* (2021), zoonotic *Yersinia* spp. recovered from wild boar only include

Y. enterocolitica and *Y. pseudotuberculosis*. Although the latter was not recovered from any of the samples, it should be taken into consideration that random colonies were chosen for MALDI-TOF testing, meaning that a larger testing could have revealed other species. *Y. enterocolitica* is a species that can be found in the gastrointestinal tract or pharynx of mammals, and which is able to grow at temperatures down to freezing point. From a food safety perspective, *Y. enterocolitica* can cause *yersiniosis*, an illness that can induce vomiting, diarrhoea, and fever for children under the age of 10 and severe abdominal pains, similar to appendicitis, for teenagers. In adults, *yersiniosis* can cause joint pain that travels around the different joints of the body. In the worst case, these pains can last for months, or up to years for some individuals. Fortunately, the vast majority of strains are avirulent, thus harmless from an infection point of view (Lindberg *et al.*, 2012). However, it should be pointed out that virulent strains can thrive in the same environments as avirulent ones, which implies that there is a possibility for the occurrence of pathogenic strains as well. Both avirulent and pathogenic strains of *Y. enterocolitica* were for example recovered by Gill (2007) from store bought wild boar meat and wild boar carcasses.

Further studies are needed to examine microflora and bacterial loads during other periods of the hunting season than winter and spring, since summer and autumn could pose challenges regarding sufficient chilling to inhibit microbial growth. Also, examining further correlations between number of days hanging and temperature effects after the harvest is suggested, as well as determining the presence of pathogenic strains found in wild boar samples such as *Y. enterocolitica.*

7. Conclusion

The results of the study suggest that the differences between samples slaughtered by hunters, local retail establishments and game handling establishments do not differ significantly other than on the aerobic bacterial load. Although, when using the hygienic criteria as a basis to determine the hygienic quality of the samples, 29 unsatisfactory samples out of 39, suggests more efforts concerning hygienic handling are needed to increase the number of *acceptable* or *satisfactory* carcasses. By increasing knowledge, concentrated efforts and cooperation with hunters, it is likely that the proportion of hygienically approved carcasses can be increased, as reported by Paulsen (2011).

Suggested actions to increase hygiene are not easily formulated as sampling and swabbing of surfaces and equipment throughout the entire supply chain was outside the scope of the study. However, a shorter hanging period of 7 days or less for carcasses seems to have affected the hunting and LRE samples positively in terms of approved or satisfactory carcasses. It appears that this trend was masked in the high number of unsatisfactory GHF samples, potentially related to the number of carcasses handled in the continuous processing line. In smaller premises where evisceration, skinning, cutting and further processing is carried out in the same areas, slaughter must not take place at the same time as other processing. It could also be recommended to thoroughly sanitise, wash and disinfect the premises before switching between operations. If possible, it would also be beneficial to have a separate section or room for grinding, slicing and vacuum packing.

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Popular science summary

The consumption and appreciation of wild boar meat has increased over the years and can be seen as a nutritional and more sustainable option compared to farmed meat. The latter is because wild game meat addresses environmental and ethical concerns raised in connection to farmed meat production. A national survey made in 2023, indicated that around 20 % of the Swedish population ate game meat on a regular basis and that about a third of that meat derived from wild boar. Previously, a majority of game meat has been sold as frozen, but game handling establishments and retailers have seen an increased demand for fresh and vacuum-packed products, where one of the most requested wild boar products is mince. Still there is a lot of wild boar meat that is only consumed privately and does not reach a broader group of consumers.

The reason for this is that wild boar meat is surrounded by stricter regulations compared to other game. Mainly, because wild boars are known carriers of pathogens such as *Trichinella* which is a parasite that can give rise to trichinosis in humans. This disease can cause painful fluid accumulations, bleeding in the eyes and allergic symptoms. The severity of conditions can vary from mild and asymptomatic to severe and fatal as the disease is hard to detect at the early stages. Thus, there have been limited ways for hunters to distribute their harvest. However, a new governmental proposal is about to be implemented as a part of the Swedish food strategy, allowing hunters with a certain hygiene education to sell small amounts of wild boar meat to consumers directly, without using a specialised wild game slaughterhouse (game handling establishment) for meat approval as is mandatory today. Several stakeholders involved in the supply chain have raised concerns about the suggestion, saying that hunters lack the proper knowledge to produce safe foods, while others state the opposite. Food poisoning related to game meat consumption is rare to non-existent according to reports from the Swedish Food Agency. However, low reporting of food poisonings in general, already discarded food products when an investigation is initiated, that only one person has been ill which does not rank as an outbreak, or that the infected person/persons do/does not seek health care affect these numbers.

Due to the conflicting opinions concerning hygiene and the upcoming legislation, 39 samples of wild boar mince collected from hunters and different slaughter establishments were examined and compared to the EU hygiene criteria for minced meat. The criteria for minced meat include limit levels for *E. coli* and aerobic bacteria given in a unit called colony forming units per gram (cfu/g) which is a common unit used to measure bacterial levels in solid foods. Cfu refers of the number of living bacteria that is able to proliferate and grow in a product.

E. coli is an indicator of faecal contamination while aerobic bacteria is a more general hygiene indicator, measuring all bacteria able to live in environments with more or less oxygen. Measuring *Enterobacteriaceae*, a bacteria family that often derives from the human intestines, animal intestines or the environment, has also been used to indicate hygiene in this project. Many bacteria belonging to *Enterobacteriaceae* can be pathogenic to humans, posing serious health hazards or causing food spoilage which is why these bacteria were examined as well.

When compared to the minced meat hygiene criteria, 29 out of 39 samples in the study were classified as hygienically '*unsatisfactory*', 6 as '*acceptabl*e' and 4 as '*satisfactory*'. Mainly due to, too high levels of the generic hygiene indicator aerobic bacteria. There was no actual difference between the hunter and establishment processed samples when looking into *E. coli* levels. Although, aerobic bacteria were significantly lower in hunter samples compared to the establishments. A few examples of bacteria that were identified in the samples, besides *E. coli*, were two types of *Yersinia* and *Hafnia alvei. Yersinia* can cause *Yersiniosis*, an illness that can induce vomiting, diarrhoea, and fever for young children and severe abdominal pains, similar to appendicitis, for teenagers. In adults, *Yersiniosis* can cause joint pain, lasting for months up to years for some individuals. Both pathogenic and non-pathogenic types thrive in the same environments. The other example, *Hafnia alvei* is a common bacteria in minced meat and grows in chilled and vacuum-packed environments. This bacteria usually do not cause illness, although a person with already impaired health conditions has an increased risk of becoming ill. Cooking the minced meat thoroughly will kill all these bacteria as well as *Trichinella*.

The result of the study is important as it can be used to show that it is possible to obtain hygienic meat from hunters as well as an established slaughterhouse. However, the total number of unsatisfactory samples suggests that hygienic improvements are needed to increase the number of satisfactory carcasses. Further studies are needed to locate the actual sources of contamination. However, a few recommendations to reduce bacterial levels for hunters is to be careful not perform evisceration, skinning, cutting and further processing at the same time if the area is small. More general advice would be not to underestimate the importance of thoroughly sanitised, washed or disinfected premises and equipment. Also, keeping a good personal hygiene by not working with the meat when being ill, washing hands properly (or if running water and soap is not available), using gloves and changing them often is important to ensure a good hygiene.

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Appendix 1

Protocol for Sample Collection of Minced Wild Boar Meat 2024

Sample type: ☐ LRE ☐ Hunter sample Number of samples: _____

<u>Contact information:</u>

Name ____

Phone:____

Email:____

Sample collected by:
☐ Anna Johansson
☐ Jonas Malmsten
☐ Other person: ____________________________________

Date of collection: ________________________________

Date of the shot: ____________________________________

Hunting area: ______________________________________

Eviscération was made: Approx. temp. (℃): ______
☐ In the forest
☐ In a barn/a lodge/ machine hall/ garage
☐ Cold room/ slaughter room
☐ Other option: ____

Carcass has been hung in a:
☐ Cold room/ slaughter room
☐ In a barn/a lodge/ machine hall /garage
☐ Hung outside
☐ Other: ____

Nr of days the game has been hung: _____ Hanging temp (°C): ____________

Date of mincing: _______ Mincing room temp (°C): _______________

Number of animals included in the sample: ______________________________

Cutting details included in the mince:

Other information (e.g. target area, hunting style, etcetera):

Approximate temp. of the sample upon collection (°C):

Average temp. of the sample after transport to the laboratory (°C):

Appendix 2

Table 4.Collected information about the hunter-, local retail establishment- and game handling establishment samples used in the study together with their final hygiene classification.

Sample	Hanging establish ment	Total number of days hanging	Temperature at hanging (°C)	pH	Other collected information	Classification according to the hygiene criterions of (EC) No 2073/2005
Hunter samples (H)						
H1	Garage	9	8-10	6.13	Organs removed lying down/or hanging. Drive hunted with dog.	Acceptable
H2	Garage	9	8-10	6.05	Organs removed lying down/or hanging. Drive hunted with dog.	Unsatisfactory
H3	1 day outside cold storage, 3 days in cold storage	4	Day 1: 8-10 Remaining days: 3	5.70	This sample included cutting details from two wild boars. One of the wild boars was shot in the stomach area. The organs were removed in the forest.	Satisfactory
H4	1 day outside cold storage, 3 days in cold storage	9	Day 1: 8-10 Remaining days: 3	5.40	The shot did not hit the wild game as it was intended to do, so a search was made. The organs were removed in the forest.	Unsatisfactory
H5	1 day outside cold storage, 3 days in	8	Day 1: 8-10 Remaining days: 3	5.73	The shot did not hit the wild game as it was intended to, therefore the game	Unsatisfactory

Sample	Hanging establish ment	Total number of days hanging	Temperature at hanging (°C)	pH	Other collected information	Classification according to the hygiene criterions of (EC) No 2073/2005
	cold storage				needed to be traced afterwards.	
H6	Garage	3	4	5.71	The game walked casually into the shooter's range without being herded by the hound. The organs were removed in a slaughter room.	Acceptable
H7	Slaughter room	10	4	5.58	Organs were removed in the slaughter room.	Unsatisfactory
H8	Garage with controlled temperatu re	7	5	5.64	The shot hit the game as intended and the organs were removed in the forest.	Satisfactory
H9	Cold storage	7	3	5.74	*Complementary information was lacking* [a]	Satisfactory
H10	Cold storage	7	3	5.79	*Complementary information was lacking* [a]	Acceptable
H11	Garage	7	5	5.69	The wild game had been hit by a car and was traced to be put down.	Acceptable
Local retail establishment samples (LRE)[b]						
Sample	**Hanging establish ment**	**Total number of days hanging**	**Temperature at hanging (°C)**	**pH**	**Other collected information**	**Classification according to the hygiene criterions of (EC) No 2073/2005**
LRE1[b]	Cold room	25	2-4	6.22	This carcass was not fully eviscerated upon arrival, the rectum, genitals were still in place.	Unsatisfactory

Sample	Hanging establish ment	Total number of days hanging	Temperature at hanging (°C)	pH	Other collected information	Classification according to the hygiene criterions of (EC) No 2073/2005
LRE2[b]	Cold room	18	2-4	5.78	Boar	Unsatisfactory
LRE3[b]	Cold room	18	2-4	6.44	Wild boars sew	Unsatisfactory
LRE4[b]	Cold room	18	2-4	5.84	Boar	Unsatisfactory
LRE5[b]	Cold room	18	2-4	5.87	Were eviscerated on the LRE premises	Unsatisfactory
LRE6[b]	In a cooled slaughter room	14	3	5.86	Delivered eviscerated	Unsatisfactory
LRE7[b]	In a cooled slaughter room	14	3	5.84	Delivered eviscerated	Unsatisfactory
LRE8[b]	In a cooled slaughter room	14	3	5.85	Delivered eviscerated	Unsatisfactory
LRE9[b]	In a cooled slaughter room	3	4	5.59	Eviscerated in slaughter room. A young wild boar. It was a head shot. Not hung for a long period, young animal. The mill had the same temperature as the cutting room (about 8 degrees).	Satisfactory
LRE10[b]	Cold room	5	4	5.60	Eviscerated in the forest and skinned in the butchery. Shot in the shoulder. Parts used in the mince included the shoulder, leg, and trim. The mill had the same temperature	Acceptable

Sample	Hanging establishment	Total number of days hanging	Temperature at hanging (°C)	pH	Other collected information	Classification according to the hygiene criterions of (EC) No 2073/2005
					as the cutting room (about 8 degrees).	
LRE11[b]	Cold room	7	Day 1: 10 Day 2-3: 6 Day 4-7: 4	5.80	Eviscerated in the forest and skinned in a lodge. The shot hit the shoulder. The mill had the same temperature as the cutting room (about 8 degrees).	Acceptable
Game handling establishment (GHE) samples						
GHE1	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.97	Collected at the start of the mincing process at 8:00. Eviscerated before delivery.	Unsatisfactory
GHE2	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.85	Collected at the start of the mincing process at 8:00. Eviscerated before delivery.	Unsatisfactory
GHE3	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.77	Collected at the start of the mincing process at 8:00. Eviscerated before delivery.	Unsatisfactory
GHE4	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.91	Collected at the start of the mincing process at 8:00. Eviscerated before delivery.	Unsatisfactory
GHE5	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.91	Collected at the start of the mincing process at 8:00. Eviscerated before delivery.	Unsatisfactory
GHE6	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.87	Collected at the start of the mincing process at 11:30.	Unsatisfactory

Sample	Hanging establishment	Total number of days hanging	Temperature at hanging (°C)	pH	Other collected information	Classification according to the hygiene criterions of (EC) No 2073/2005
					Eviscerated before delivery.	
GHE7	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.86	Collected at the start of the mincing process at 11:30. Eviscerated before delivery.	Unsatisfactory
GHE8	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.79	Collected at the start of the mincing process at 11:30. Eviscerated before delivery.	Unsatisfactory
GHE9	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.85	Collected at the start of the mincing process at 11:30. Eviscerated before delivery.	Unsatisfactory
GHE10	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.90	Collected at the start of the mincing process at 11:30 Eviscerated before delivery.	Unsatisfactory
GHE11	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.90	Collected at the start of the mincing process at 14:00. Eviscerated before delivery.	Unsatisfactory
GHE12	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.83	Collected at the start of the mincing process at 14:00. Eviscerated before delivery.	Unsatisfactory
GHE13	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.78	Collected at the start of the mincing process at 14:00. Eviscerated before delivery.	Unsatisfactory
GHE14	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.92	Collected at the start of the mincing process at 14:00	Unsatisfactory

Sample	Hanging establish ment	Total number of days hanging	Temperature at hanging (°C)	pH	Other collected information	Classification according to the hygiene criterions of (EC) No 2073/2005
					Eviscerated before delivery.	
GHE15	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.77	Collected at the start of the mincing process at 14:00. Eviscerated before delivery.	Unsatisfactory
GHE16	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.80	Collected at the start of the mincing process at 14:00. Eviscerated before delivery.	Unsatisfactory
GHE17	Cold room	Between 3-4 [c]	3-4 (maximum of 7) [d]	5.80	Collected at the start of the mincing process at 14:00. Eviscerated before delivery.	Unsatisfactory

[a] The samples were delivered to the sample handler via an agent, therefore information about placement of shot, evisceration location was lacking.
[b] The table uses the term Local retail establishments as defined in (EC) No 853/2004 to describe establishments that has been wrongfully registered for wild boar meat handling, or establishments with similar traits of an actual local retail establishment but handles wild boar meat unregistered (illegal).
[c] As it is a continuous flow of wild game, the GHEs strive to not let a carcass hang for more than an average period of 3-4 days.
[d] The sample supplier has a contract that guarantees that the carcasses will not be stored at temperatures above 7 °C; the average temperature of the cold rooms were between 3-4 °C.

Appendix 3

Table 5. Counts of total mesophilic aerobic bacteria, E. coli and Enterobacteriaceae detected in hunter samples (H), local retail establishment samples (LRE) and game handling establishment samples (GHE) presented as log cfu/g. Identified Enterobacteriaceae found in the samples are also presented.

Sample	**Total mesophilic aerobic bacteria**	**Compliance to the hygienic criteria span m–M**	***E. Coli*** [a]	**Compliance to the hygienic criteria span m–M**	***Enterobacteriaceae*** [a,b]	**Identified *Enterobacteriaceae* genera**
H1	6.3	Middle	2.1	Middle	4.7	*Hafnia alvei*
H2	7.4	Above	2.1	Middle	4.1	*Hafnia alvei*
H3	4.9	**Below**	<5	**Below**	5.1	*Hafnia alvei, Escherichia coli*
H4	6.4	Middle	4.1	Above	5.3	*Hafnia alvei*
H5	6.0	Middle	3.8	Above	5.3	*Hafnia alvei*
H6	5.9	Middle	<5	**Below**	3.6	*Hafnia alvei, Escherichia coli, Yersinia enterocolitica*
H7	7.7	Above	<5	**Below**	3.4	*Pantoea agglomerans, Serratia liquefaciens*
H8	5.0	**Below**	<5	**Below**	3.2	*Leclercia adecarboxylata, Klebsiella oxytoca, Pantoea septica*
H9	5.3	**Below**	<5	**Below**	<10	*Hafnia alvei, Serratia liquefaciens*

Sample	Total mesophilic aerobic bacteria	Compliance to the hygienic criteria span m–M	*E. Coli* [a]	Compliance to the hygienic criteria span m–M	*Enterobacteriaceae* [a,b]	Identified *Enterobacteriaceae* genera
H10	5.9	Middle	0.7	**Below**	3.1	*Pantoea agglomerans, Serratia fonticola, Buttiauxella gaviniae*
H11	6.6	Middle	<5	**Below**	4.7	*Hafnia alvei, Pantoea agglomerans*
Bacterial average	6.1		3.3		4.0	
LRE1[c]	8.1	Above	2.1	Middle	4.7	*Hafnia alvei, Serratia liquefaciens, Serratia fonticola*
LRE2[c]	7.0	Above	2.5	Middle	4.1	*Hafnia alvei, Serratia liquefaciens, Pseudescherichia vulneris*
LRE3[c]	7.5	Above	3.1	Above	5.1	*Escherichia coli, Serratia liquefaciens*
LRE4[c]	8.1	Above	2.9	Above	5.3	*Escherichia coli, Serratia liquefaciens*
LRE5[c]	7.0	Above	3.6	Above	5.3	*Escherichia coli*
LRE6[c]	8.3	Above	3.3	Above	3.6	*Escherichia coli, Serratia liquefaciens*
LRE7[c]	8.3	Above	2.0	Middle	3.4	*Escherichia coli, Serratia liquefaciens, Buttiauxella gaviniae*
LRE8[c]	8.2	Above	2.3	Middle	3.2	*Serratia fonticola*
LRE9[c]	5.0	**Below**	<5	**Below**	<10	Low levels prevented identification
LRE10[c]	6.1	Middle	1.0	**Below**	3.1	*Klebsiella oxytoca, Leclercia adecarboxylata, Enterobacter bugandensis, Citrobacter gilleni*
LRE11[c]	6.3	Middle	2.6	Middle	3.3	*Escherichia coli, Serratia fonticola, Klebsiella oxytoca, Kalamiella piersonii*
Bacterial average	7.3		2.4		3.8	
GHE1	8.2	Above	1.7	**Below**	5.1	*Hafnia alvei, Yersinia enterocolitica, Yersinia kristensenii*
GHE2	7.7	Above	2.4	Middle	4.5	*Hafnia alvei, Yersinia kristensenii*
GHE3	8.1	Above	2.6	Middle	4.9	*Hafnia alvei, Serratia fonticola*

Sample	Total mesophilic aerobic bacteria	Compliance to the hygienic criteria span m–M	*E. Coli* [a]	Compliance to the hygienic criteria span m–M	*Enterobacteriaceae* [a,b]	Identified *Enterobacteriaceae* genera
GHE4	7.5	Above	2.1	Middle	3.7	*Hafnia alvei, Yersinia kristensenii*
GHE5	8.1	Above	1.8	Middle	3.7	*Hafnia alvei, Yersinia enterocolitica, Yersinia kristensenii*
GHE6	7.6	Above	2.0	Middle	4.4	*Hafnia alvei, Yersinia kristensenii*
GHE7	7.4	Above	3.3	Above	4.3	*Hafnia alvei, Escherichia coli, Yersinia kristensenii*
GHE8	7.5	Above	1.9	Middle	4.7	*Hafnia alvei, Yersinia kristensenii*
GHE9	7.5	Above	2.3	Middle	4.9	*Hafnia alvei, Yersinia enterocolitica*
GHE10	7.5	Above	1.0	**Below**	4.4	*Hafnia alvei, Yersinia kristensenii, Buttiauxella gaviniae*
GHE11	8.1	Above	2.1	Middle	4.2	*Hafnia alvei, Escherichia coli*
GHE12	8.3	Above	1.7	**Below**	4.6	*Hafnia alvei*
GHE13	7.5	Above	1.9	Middle	4.6	*Hafnia alvei*
GHE14	8.0	Above	2.0	Middle	5.0	*Yersinia enterocolitica, Yersinia kristensenii*
GHE15	7.6	Above	2.4	Middle	4.8	*Hafnia alvei*
GHE16	7.6	Above	2.3	Middle	4.8	*Hafnia alvei, Klebsiella pneumoniae*
GHE17	7.3	Above	1.9	Middle	4.2	*Hafnia alvei*
Bacterial average	7.7		2.1		4.5	

[a] For calculation of average values bacterial levels of <5 log cfu/g and <10 log cfu/g was translated to 2.5 and 5 log cfu/g respectively.

[b] No hygienic processing criteria for minced meat are set for *Enterobacteriaceae* in regulations (EC) No 2073/200.

[c] The table uses the term Local retail establishments as defined in (EC) No 853/2004 to describe establishments that has been wrongfully registered for wild boar meat handling, or establishments with similar traits of an actual local retail establishment but handles wild boar meat unregistered (illegal).

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