# Bacterial contamination on pork necks and loins

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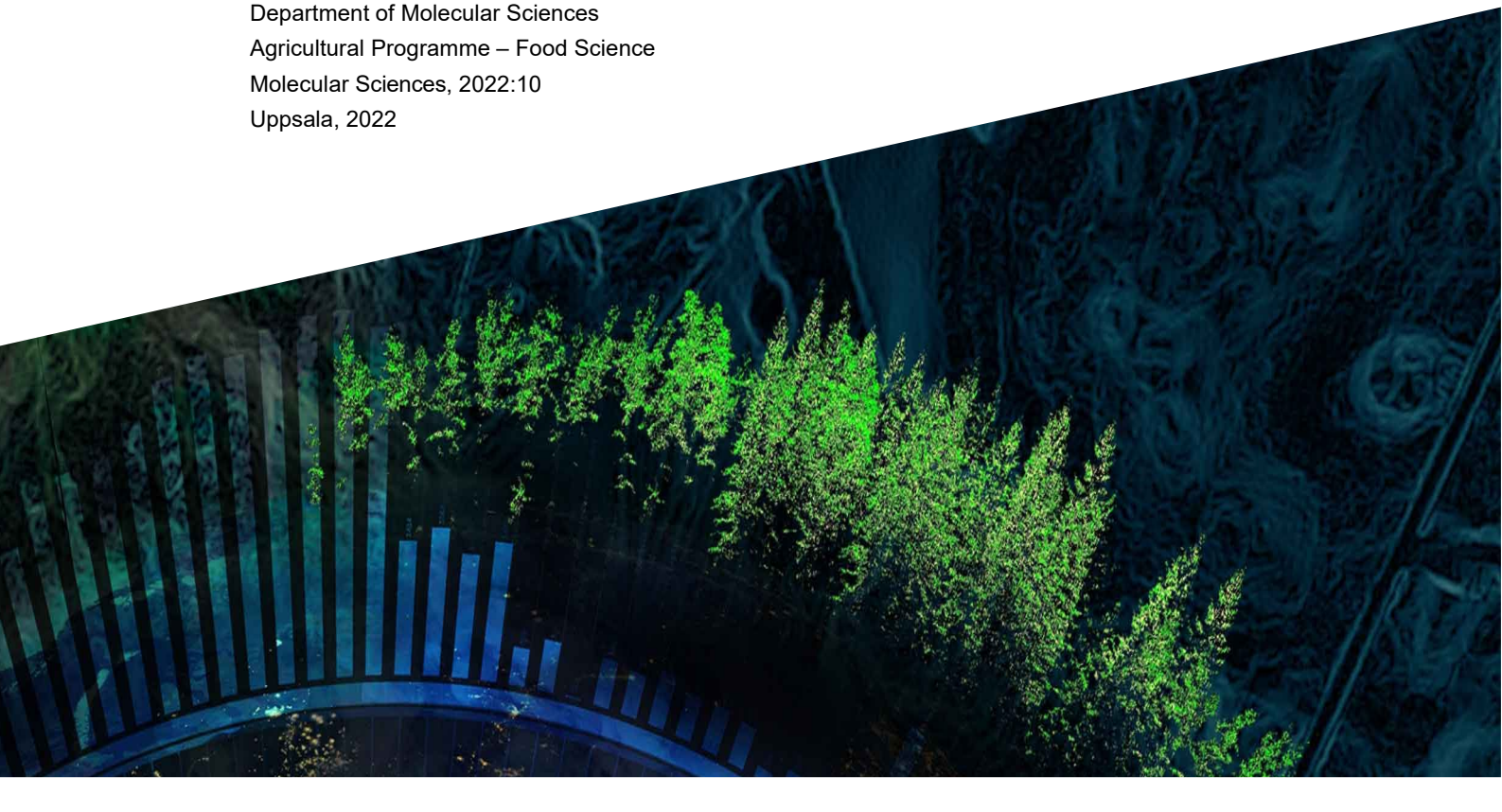

# Bacterial contamination on pork necks and loins

*Förekomsten av bakterier på fläskkarré och kotletter*

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## Abstract

The aim of the study was to investigate if there is a difference in the quantity of bacteria that could affect the shelf life of pork loins and pork necks. Samples from pork loins and pork necks from a cutting plant was analysed during three occasions during three weeks. The samples were collected during January to February 2022 and analysed regarding the total number of aerobic bacteria on PCA agar and bacteria belonging to family *Enterobacteriaceae* on VRBG agar plates. The average amount of aerobic bacteria was 2.9 log CFU/g in loins and on pork necks 3.3 log CFU/g, though this was not statistically signifacant. Bacteria belonging to family *Enterobacteriaceae* were below the detection limit of <1.0 log CFU/g on all loins and necks except from one sample of pork necks and one from loins where 1.0 log CFU/g were quantified. Levels of aerobic bacteria on fresh carcasses are deemed accepted <4.0 log CFU/g and for *Enterobacteriaceae* <2.0 log CFU/g according to 2001/471/EC. Isolated bacteria were identified by MALDI-TOF which identified R*othia.endophytia, Escherichia coli, Acinnetoacter guillouiae, Massilia oculi, Psedumonas fragi* and *Staphylococcus capitis*. *P. fragi* which are commensals in the skin and eyes from humans and some of them are also well-known spoilage bacterium. The amount of aerobic bacteria found on the pork necks appeared to be slightly highly compared with the samples from loins, though this difference was not significant. However, it might indicate that the shelf life for loins should be longer compared to pork necks.

Syftet med studien var att undersöka eventuella skillnader i förekomsten av bakterier på karré och kotlett av fläsk vilket kan påverka hållbarheten på produkterna. Prover från kotletter och karréer togs vid tre tillfällen under tre veckors tid under januari till februari 2022 från en styckningsanläggning och analyserades avseende total antal aeroba bakterier på PCA agar och bakterier tillhörande familjen *Enterobacteriaceae* på VRGG agar. På kotletterna påvisades i medeltal 2,9 log CFU/g och på karréerna påvisades 3,3 log CFU/g aeroba bakterier dock inte statistiskt signifikant. Bakterier tillhörande familjen *Enterobacteriaceae* kunde endast kvantifieras från ett prov från fläskkarrée, (1,0 log CFU/g), och ett från en kotlett, (1,0 log CFU/g). Övriga prov hade färre än 1,0 log CFU/g vilket var detektionsgränsen i studien. Ett flertal bakterier identifierades med hjälp av MALDI-TOF vilket resulterade till *Rothia endophytia, Escherichia. coli, Acinetoacter guillouiae, Massilia oculi, Psedumonas fragi* and *Staphylococcus capitis. P. fragi* som är vanligt förekommande i huden hos människa, vissa av dem är också kända för att förstöra kött. Sammanfattningsvis påvisades fler bakterier på karrén än på kotletterna även om skillnaden inte var signifikant, men det kan indikera att hållbarheten på kotlett är något längre än karrén.



*Keywords:* Pork meat, Pork loins, Pork necks, chops, food safety, food control, contamination, aerobic bacteria, *Enterobacteriaceae*

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# Abbreviations

| | |
|---|---|
| CFU | Colony Forming unit |
| LAB | Lactic acid bacteria |
| MAP | Modified Atmosphere Packaging |
| MALDI-TOF MS | Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry |
| PCA | Plate count agar |
| PSE | Pale, Soft and Exudative |
| VRBG | Violet Red Bile Glucose agar |

# Introduction

## 1.1 Slaughtering and cutting process

When considering bacterial contamination of pork, primary sources can be the pig itself as well as the slaughtering environment and handlers. Therefore, a description of the entire slaughtering and cutting process is given here. The slaughtering process starts with the live animal arriving to the abattoir and they are kept in the stables before the stunning. Larger abattoirs use $CO_2$ to stun the pigs in an elevator that goes underground where it is filled with $CO_2$. The pig suffocates, this elevator used is usually referred to as a Butina after the company that produce them. Smaller abattoirs use a tong with electric current to stun the pigs. The Butina is usually the preferred method of stunning as there are more accidents happening, such as broken vertebra's (Wotton *et al.*, 1992) with electrocution as well as more Pale, Soft and Exudative (PSE) pigs due to increased stress levels caused from more interaction with humans.

After stunning the deblooding is performed by sticking a knife into the animals throat. This must be done 60 seconds after the use of the suffocation technique or 20 seconds after the use of the electrocution technique according to the Swedish regulation (Jordbruksverket. 2022). After the deblooding, the animal is dead and is now considered as a carcass and not an animal. When the pig is killed the pH of the meat decrease from around 7 to 5.4-5.5 due to glycogen being converted into lactic acid. Sometimes if the muscles are extensively stimulated before death the meat can become "stressed", usually called PSE, which in practice means that the pH of the meat is lower than the usual pH 5.4-5.5. The result is that the meat will have increased cooking losses and be less juicy (Adams & Moss, 2008).

After the deblooding the pigs are scalded either by hot water or steam, the temperature of the water/steam and time differs between abattoirs depending on what equipment is used and the size of the machines, but it is usually around 85°C and around 15-30 minutes. This procedure will loosen up the hairs on the skin and most of them will fall off. Some abattoirs have whipping machines that remove more hair before the flaming. However, some abattoirs just let the pigs go into the flaming machine first and then let the whipping machines take care of the left-over burnt hair and soot. In some cases where the machinery is not state of the art there might have to be some manual scraping to remove the last of the soot and hair. The flaming creates a clean surface on the skin and the skin can now be

considered as food, it also makes the skin in a yellowish color which looks more appetible.

An incision is made by opening the abdomen either by machine which all large abattoirs are using or by hand which most middle to small sized abattoirs do. The breast bone can be opened either by saw or by knife going through the soft bone. A saw is preferred since the ribs will look much better after the cutting process. The rectum is cut around and sometimes a bag is put over the anus and rectum to avoid contamination of feces to the inner parts of the pigs. The stomach is removed together with the esophagus. Skilled slaughtermen make sure the gallbladder is taken out clean from the liver to increase the yield in weight of the liver. After this the organs are removed in one package as kidneys, diaphragm, liver, lungs and tongue. Removal of the toungue is a job that also require skilled slaughtermen as the tonsils should be removed with the toungue to lower the risk of spreading *Yersinia entercolitica* (Livsmedelsprovtagning. 2022). The carcass is after this cleaved by either a machine with rotating blades or a saw that is operated by a person. The saw could produce saw dust that can stimulate the growth of bacteria and lower the shelf life of the cuts or slices in the end. Another method can be an axe but that requires a lot of skill to have it the carcass cleaved evenly and also work intensive to do it, so only very small abattoirs use an axe. The head is loosened at the atlas, but still hanging on the carcass until the pig is weighed since the farmer will get paid for the head as well. Therefore, it is just loosened so it is easy to remove later once the pig is cooled down. One reason for loosening the head is that the meat gets harder once it is cooled and it may be difficult to remove then. The lard is removed either by hand or machine. Then the trimming of the carcass is done usually by knife or by vacuum removing remaining lard and other trimmings left. After the pig is weighed, the front feet and head is removed. Pigs are usually just cooled over night to reach a temperature of <7°C (Livsmedelverket. 2022).

The cutting is taken place mostly the day after the slaughter process. The pig is usually cut into three pieces, shoulder that includes the neck, middle part that includes the loin, and ham. It's usually parted by cutting between the sixth and seventh rib, and through the second soft bone in the spine from the ham. There are many different ways to cut a pig but here traditional Swedish cuts are explained. Although, before the parting the tenderloin is usually removed and trimmed. On the shoulder it should be sawed across the ribs from the tip of the small triangle under the neck to as close as possible to the last rib of the neck. At this point the neck is cut of following the membrane to the shoulder blade then cutting through the meat and following above the fat, it may be deboned. The ribs are cut off following the membrane all the way, they are trimmed to the factory standards after. Cuts are made along the shoulder blade and it is pulled out by hand and trimmed. The front leg is sawed off. The chin is cut off. The shoulder is deboned and trimmed depending on how it will be used. The middle part is sawed from the tip of the triangle under the bones of the loin across the ribs. The loin can be just cut off as loin with bone and skin or the skin and fat can be removed, the loin can be either sold with bone or be deboned and sold as boneless. The pork belly is usually deboned and the bones are sold as spareribs and the pork belly can either

be sold with or without the skin. For the ham, firstly the pelvis bone is removed and trimmed, then the back leg is cut off. A cut along the lower part of the topside is made and the femur bone is removed. Then the ham is cut into either steaks or clean cuts which are trimmed. All trimmings on the pig are taken care of and are used for minced meat or sausage.

## 1.2 Bacteria

Common spoilage bacteria on pork are *Brochatrix thermosphacta, Pseudomonas* spp*., Carnobacterium* spp*.,* bacteria belonging to family *Enterobacteraceae, Lactobacillus* spp., *Leuconnostoc* spp*.* and *Shewanella putrefaciens* (Borch, Kant-Muermans and Blixt, 1996). Some are more prevalent in vacuumed packed and modified atmosphere packaging (MAP). Lactic acid bacteria (LAB) are more proned to cause spoilage due them being anaerobic. *Brochatrix* spp. can be a spoiler under certain conditions if the meat is vacuumed pack so can also *Clostridium* spp*.* (EFSA, 2016). The meat is assessed as spoiled when the total amount of bacteria reaches log 7 per $cm^2$ (Adams & Moss, 2008). The total amount of aerobic and *Enterobacteriaceae* measured on carcasses (Table 1) is a way to know limits where to know where an accepted level of contamination starts on cuts.

*Table 1: Daily mean values for marginal and unacceptable results for bacterial performance criteria for pig (log cfu/cm2) for samples taken by the destructive method.*

| | Acceptable (log CFU/$cm^2$) | Marginal range (log CFU/$cm^2$) | Unacceptable (log CFU/$cm^2$) |
|---|---|---|---|
| Aerobic bacteria | <4.0 | 4.0–5.0 | >5.0 |
| *Enterobacteriaceae* | <2.0 | 2.0–3.0 | >3.0 |

On a survey mapping of bacteria on pig carcasses in Sweden a mean of 3.5 log CFU/$cm^2$ of aerobic bacteria and 0.3 log CFU/$cm^2$ of bacteria belonging to Family *Enterobacteriaceae* was found on pig carcasses after the slaughtering process (Lindblad, 2006).

### *1.2.1 Food spoilage bacteria*

*Pseudomonas* spp. are non-spore forming gram-negative rods. They are motile due to one or more flagella. *Pseudomonas* are facultative anaerobes, since they can exploit $NO_3$- as final electron acceptor in the respiratory chain (Hossain, 2014). *P. fragi* is among those that can be found in meat and can grow between 0-35°C and

since meat is stored 0-4°C most of the time it still can grow there (Hebraud *et al.*, 1994).

Family *Enterobacteriaceae* is a large family of bacteria with about 50 genus. They are commensal in the intestinal microbiota of humans as well as animals and presence of *Enterobacteriaceae* indicate a not sufficient personal hygiene or fecal contamination. In common they are facultative anaerobic and not spore forming. Bacteria such as *Salmonella, Hafnia alvei, E. coli, Shigella* spp*., Y. enterocoltica* belong all to family *Enterobacteriaceae* (VetBact 2022). *Eschericha coli* is normally not considered as a pathogen, but there are strains of *E. coli* that are pathogenic for example *E. coli* O157:H7 which could cause bloody diarrhea and hemolytic uremic syndrome (Nguyen and Sperandio, 2012).

*Carnobacterium* is a genus which contains nine species and two of them are usually found in food, those are *C. divergens* and *C. maltaromaticum.* It usually grows at lower temperature and anaerobically which indicates it is more of an issue with vacuum packed and MAP products. *Carnobacterium* is also a LAB. In meat *Carnobacterium* is able to grow as low as -1.5°C (Leisner *et al.*, 2007).

*Leucostoc* is a LAB which are hetero fermentative and are also present in other food stuff like sauerkraut and kombucha. It also seem to be more of an issue with anaerobic environments like vacuum packed and MAP (Haikara and Helander, 2006).

*Shewanella* spp. is mostly associated with fish but can also be found in red meat even though it prefers a higher pH and protein rich environments. Although it seems more of an issue in anaerobic environments like vacuum packed or MAP (Satomi, 2014).

*Acinetobacter guillouiae* is a mesophilic, gram negative, non-motile and catalase positive bacteria. It is considered a human pathogen due to appearing in infected wounds (Podstawka. 2022). *Acinetobacter* is also described as a spoilage bacterium for beef which is also classified as red meat (Adams & Moss, 2008).

### 1.2.2 Other bacteria

*Kocuria rhizophilia* is a bacteria found on humans and is a cocci, gram positive, non-motile, aerobic bacteria. Due to its small genome, it has a lot of industrial applications (bacmap.wishartlab.com, 2022).

*Rothia endophytica* is found in the human gut flora and is an aerobic, gram positive, mesophilic bacteria (Podawska, 2022)

*Aeromonas salmonicidia* is a gram negative, non-spore forming, non-motile, rod and occasionally as cocci. It is a pathogen for fish but nonpathogenic for humans. It is usually spread by unclean water (Laboratoy, 2022).

*Massilia oculi* is a rodshaped and grows at 15-37°C at pH 5.5-10.5. It was isolated from the human eye and there have been single cases where it has been pathogenic (Podawska, 2022).

*Staphylococcus capitis* is a mesophilic, cocci that grows at 37°C. It can be found on humans in the hair, face, neck, scrotum, and ears. It is not pathogenic (Schleifer and Kloos, 1975).

## 1.3 Aim of the study

The aim of the study was to evaluate the difference in the amount of bacteria on pork loins and pork necks to get an opinion if it could affect the shelf life of the product. The hypothesis is that a higher amount of bacteria will be isolated from pork necks.

# 2 Materials and Methods

## 2.1 Sampling

Samples was taken at a cutting plant on Mondays 31/1, 7/2 and 14/2 2022 from pigs that had been slaughtered last Friday. At each sampling occasion ten samples á 10g was taken from the lower part of the neck and ten samples á 10g samples was taken from the loin closest to the ham. All samples were put in stomacher bags and kept in chilled Styrofoam boxes until arrival at the lab.

## 2.2 Aerobic bacteria

The samples were analyzed for total number of live, aerobic bacteria according to NMKL-method 86 (5th Ed., 2013). In brief, 90 mL buffered peptone water (BPW) were added to the 10 g meat and stomached (easyMIX Lab Blender, AES-Chemunex, Weber Scientific, Hamilton, NJ, USA) for one minute. A 10-fold serial dilution in 0.1% (v/v) peptone water (Oxoid, Basingstoke, UK) was prepared and 1.0 mL from each dilution was mixed carefully with 10-15 mL of plate count agar (PCA) (Oxoid, Basingstoke, UK) in a Petri dish (9 cm diameter). After agar solidification, the plates were incubated at 30.0°C for 72±7 hours. Selected colonies of interest were spread upon beef blood agar and incubated at 24±3 hours at 37°C. MALDI-TOF was used to determine what species was found. A total of 18 samples was prepared for MALDI-TOF.

All colonies were counted on the plates and the number of bacteria was quantified according to ISO 7218:2007/A1:2013 and expressed as log colony-forming units (CFU) per gram.

The results were analyzed by T-test for two independent means, performed using a statistical program on the Internet website "Social Science Statistics" (www.socscistatistics.com, 2022). The tests verified the difference in bacteria from loin and neck. A probability level of $p<0.05$ was considered statistically significant.

**Standard Formula** (ISO 7218:2007/A1:2013)

**$N = \Sigma C / (V \times 1.1 \times d)$**

- *N* no. micro-organisms
- *ΣC* sum of the colonies on 2 plates from successive dilutions
- *V* volume of the inoculum/plate, in ml
- *d* 1st countable dilution retained
- *1.1* a factor used when the so-called weighed mean is calculated from two plates (if only one plate is used, the factor should be 1.0 and for three plates 1.11)

## 2.2 Analysis of *Enterobacteriaceae*

The samples were analyzed for *Enterobacteriaceae* according to method NMKL 144, 3. Ed., 2005. In brief, 10 g of sample were transferred to nine times the volume (approx. 90 mL) of peptone water (BPW) and homogenized in a stomacher (easyMIX Lab Blender, AES-Chemunex, Weber Scientific, Hamilton, NJ, USA) for one minute. A 10-fold serial dilution in 0.1% (v/v) peptone water was prepared and 1.0 mL from each dilution was mixed carefully with 10-15 mL of violet red bile glucose agar (VRBG) (Becton, Dickinson and Company, Sparks USA) in a petri dish (9 cm diameter), with a final overlay of an additional 5 mL VRBG. After agar solidification, the plates were incubated at 37°C for 24±3 hours. Dark purple colonies 1-2 mm in diameter and surrounded by a purple halo were included in presumptive counts. Colonies preliminarily identified as *Enterobacteriaceae* were cultured on blood agar and incubated at 37°C for 24±3 hours. The identity of the colonies was verified by oxidase testing. Oxidase-negative colonies were identified to species level using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) (Brucker Daltonics, Bremen).

# Results

## 3.1 Total number of aerobic bacteria

Samples from 31st of January show a mean of 3.2 log CFU/g on loins and 3.2 log CFU/g on necks and on the 7th of February 3.0 log CFU/g on loins and 3.2 log CFU/g on necks. The samples that were collected the 14th of February showed the lowest mean of of 2.3 log CFU/g on loins and 3.0 log CFU/g on necks (Figure 1).

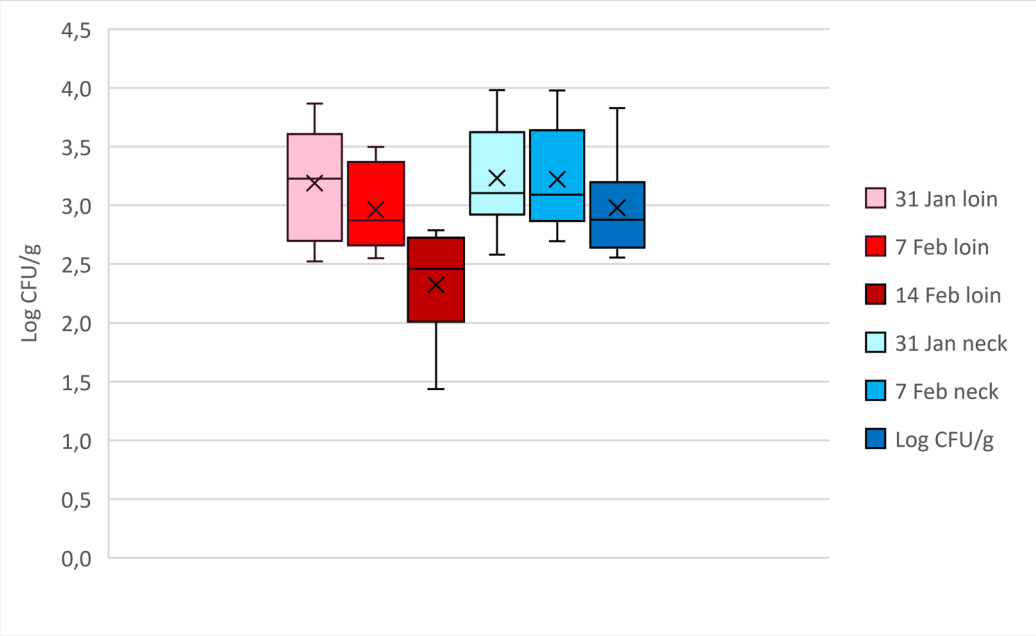


Figure 1: Number of aerobic bacteria from loin and neck meat from pigs by test occasion. Boxes show values between the 25th and 75th percentiles

A difference was found regarding the total number of aerobic bacteria isolated from loin and neck meat from the pigs. The mean of the 30 loins was 2.8 log CFU/g and on the 30 necks 3.1 log CFU/g (Figure 2).

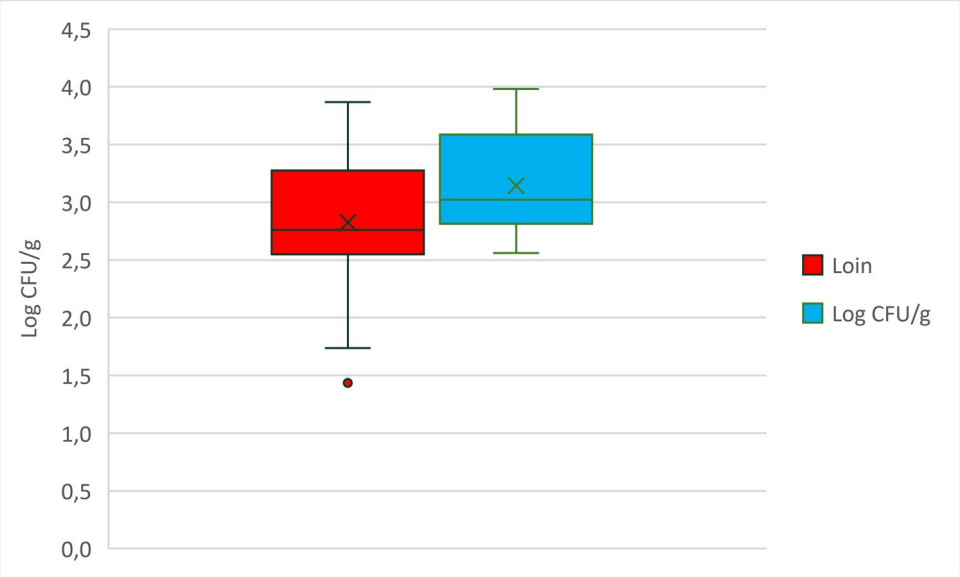

Figure 2: Number of aerobic bacteria from loin and neck meat from pigs. Boxes show values between the 25th and 75th percentiles.

The P-value was calculated to 0.49 which is not significant at $P<0.05$.

## 3.2 Bacteria belonging to Family *Enterobacteriaceae*

Only two colony of suspected *Enterobacteriaceae* was found on VRBG, one the 31st of January on loins and the other on 7th of February on necks. No bacteria suspected to be *Enterobacteriaceae* was found on the 14th of February. Both colonies were negative for oxidase tests which confirmed them to be *Enterobacteriaceae*. A mean of -0.5 log CFU/g was found, so it would be <1 log CFU/g.

## 3.3 MALDI-TOF MS

Colonies of interest from the PCA plates was recultured on blood agar plates and incubated for 24±3 hours. The isolates were analyzed by MALDI-TOF MS.

The two colonies found at the VRBG plates were recultured on blood agar plates and incubated for 24±3 hours. Growth of pure culture was observed on both and they were identified by MALDI-TOF MS to *Escherchia coli* and *Hafnia alvei* (Table 3).

*Table 2: Bacteria isolated from PCA and identified by MALDI-TOF MS.*

| Date | Sample | Bacteria |
|---|---|---|
| 31-jan | Loin | *Pseudomonas fragi* |
| 07-feb | Loin | *Rothia endophytica* |
| 07-feb | Loin | *Acinetobacter guillouiae* |
| 07-feb | Loin | *Rothia endophytica* |
| 07-feb | Neck | *Aeromonas salmoncidia* |
| 14-feb | Neck | *Acinetobacter guillouiae* |
| 14-feb | Neck | *Pseudomonas fragi* |
| 14-feb | Neck | *Massilia oculi* |
| 14-feb | Neck | *Staphylococcus capitis* |

*Table 3: Bacteria isolated on VRBG plates and identified by MALDI-TOF MS plates.*

| Date | Sample | Bacteria |
|---|---|---|
| 31-jan | Neck | *Escherchia coli* |
| 07-feb | Loin | *Hafnia alvei* |

# Discussion

## 4.1 Aerobic bacteria

More bacteria were found on samples from necks compared with loins on all occasions except for the first sample on the 31st of January. This may be because of inexperience and not wiping the agar bottles properly. Furthermore, there are some inconsistent results from this date (appendix 1), but experience was gained during the project and the other occasions more consistent results were received. The mean of 2.8 log CFU/g on loins and 3.1 log CFU/g on necks support the original hypothesis that more bacteria would be found on the necks, although it was not statistically significant it might be a factor resulting in a lower shelf life in the end. But the amount of bacteria is in agreement with previous studies from carcasses that found a mean of around 3.5 log CFU/$cm^2$ (Lindblad, 2006).

It was not unexpected finding lower numbers of CFU/g in this study since sampling in this study were performed on in a cold environment and not fresh carcasses at the abattoir. Although since the accepted levels of <4.0 CFU/g according to (Lindblad, 2006), the cuts from the pork can be deemed as good with low bacteria and good shelf life.

## 4.2 *Enterobacteriaceae*

First week one colony belonging to family *Enterobacteriaceae* was found on one sample of loin and on the second week one colony was found on one sample of neck. The mean of -0.5 log CFU/g is very small and another study on fresh carcasses was 0.3 log CFU/$cm^2$ which was also very small although it was performed on fresh carcasses the lower value is to be expected (Lindblad, 2006) , which shows that fresh cuts in theory should have lower fecal contamination. These results indicate that the fecal contamination was almost negligible in this study and does not seem like an issue for this cutting plant.

## 4.3 MALDI-TOF

The bacteria found and identified by MALDI-TOF was mostly bacteria non-pathogenic bacteria that occur in the environment and most likely connected to the commensal human and pig flora together with *R. endophytia, E. coli, A. guillouiae, M. oculi* and *S. capitis* that relate to the human skin, eyes, and gut flora which was expected (Schleifer and Kloos, 1975). *P. fragi* belonging to the *Pseudomonas* family was also expected to isolated since it is a well-known spoilage bacterium and probably cannot be avoided. These findings are not in agreement to the bacteria found in other similar studies (Borch, Kant-Muermans and Blixt, 1996). This may be because of the other studies included packaged products instead of fresh cuts. Although, in another study on pig skins before chilling *Acinetobacter* spp., *Streptococcus* spp. and *Pseudomonas* spp. was found similar to this study (Feldhusen, Woltering and Fries, 1992). A study following the entire progress of the pig should most likely find *Brochatrix* spp., *Acinetobacter* spp. and *Pseudomonas* spp. (Gill and Bryant, 1992). It was possible that *Brochatrix* spp. was present in this study although it was not found

The two colonies identified on the VRBG plates were *H. alvei* and *E. coli*. Both are part of the microbiota in the intestines
of humans and animals and therefore an expected finding. Since it was such a low number of bacteria on the VRBG plates, it should not be a big issue as well as they are non-pathogenic.

## 4.4 Improvements

It was a bit of a challenge to find a cutting plant for pigs in the Uppsala area that had enough pigs to sample. Of course, more cutting plants included in the study would most likely improve the results. Also, small scale slaughter houses and cutting plants which kill and cut pigs in the same facility would be very interesting to include in the study. It should also had been of interest to compare different seasons since from personal experiences, necks tend to have much lower shelf life in the summer compared with in the winter. The sampling in this study were performed during the winterperiod with a temperature below zero and snow was present during all three weeks.

## 4.5 Conclusion

The hypothesis of this study was that there are more bacteria on necks than on loins and this was also shown since the mean number of CFU/g on the necks were higher compared with the loins. The identified bacteria by MALDI-TOF MS were no real surprises since the majority of the bacteria found are known as environmental

bacteria and most likely connected with contamination from humans or the pigs. Common food spoilage bacteria like *Pseudomonas* and *Acinetobacter* were found on both loins and necks which was expected and *P. fragi* is a devious bacterium for meat since it grows at 0°C.

This study was done on fresh cuts from chilled carcasses and a comparison to other studies are not suitable since they mostly use fresh carcasses direct from the abattoir and/or packaged cuts. The higher amount of bacteria on the pork neck could be due to many reasons such as the neck hanging closer to the floor during cooling. Seasons could be another big reason for more bacteria in the pork neck which result in a lower shelf life. In the summer it is hot outside and moisture forms inside the chilling rooms and drops to the floor increasing relative humidity and thus giving the bacteria more water to increase activity.

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# Popular science summary

Shelf life of meat is an important aspect of products, and it is important for the grocery stores to be able to store products for as long as possible as well as the consumer to be able to store in their fridge as long as possible.
This study included pork necks and pork loins to compare the number of bacteria present on them. The hypothesis was that there were more bacteria present on the necks thus it is more often necks have shorter shelf life. This is due to many reasons, but one is that the neck hangs closer to the floor before making cuts of the pig carcass.

There were more bacteria was found on the pork necks with a mean of 3.1 log CFU/g and on pork loins with a mean of 2.8 log CFU/g, though this was not significantly different. This could be one of the reasons that necks have lower shelf life than loins. The bacteria present was identified as bacteria being present in the environment and commensal bacterial flora in humans and animals. There were also well-known spoilage bacteria by the name of *Pseudomonas fragi* present. Although low levels were detected the food is deemed as safe to eat and none of the bacteria found was pathogenic and all tests was well below of accepted levels.

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# Appendix 1

*Appendix 1: Results on PCA plates from 31th of January*

| 31-jan Loins | PCA | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ |
|---|---|---|---|---|
| 1 | | 28 | 10 | 1 |
| 2 | | 176 | 19 | 2 |
| 3 | | 30 | 5 | 2 |
| 4 | | 55 | 6 | 1 |
| 5 | | 275 | 30 | 3 |
| 6 | | 95 | 9 | 0 |
| 7 | | 134 | 35 | 9 |
| 8 | | 267 | 93 | 31 |
| 9 | | 299 | 262 | 111 |
| 10 | | 383 | 317 | 117 |

| Necks | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ |
|---|---|---|---|
| 1 | 121 | 175 | 122 |
| 2 | 452 | 199 | 1 |
| 3 | 317 | 18 | 3 |
| 4 | 746 | 55 | 264 |
| 5 | 88 | 27 | 3 |
| 6 | 123 | 12 | 9 |
| 7 | 65 | 41 | 3 |
| 8 | 40 | 2 | 0 |
| 9 | 49 | 8 | 0 |
| 10 | 93 | 43 | 2 |

# Appendix 2

*Appendix 2: Results from PCA plates on the 7th of February*

| 07-feb Loins | PCA | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ |
|---|---|---|---|---|
| 1 | | 238 | 11 | 1 |
| 2 | | 75 | 5 | 1 |
| 3 | | 126 | 12 | 0 |
| 4 | | 271 | 14 | 4 |
| 5 | | 36 | 3 | 0 |
| 6 | | 50 | 7 | 0 |
| 7 | | 50 | 3 | 1 |
| 8 | | 314 | 29 | 5 |
| 9 | | 37 | 4 | 1 |
| 10 | | 76 | 8 | 0 |

| Necks | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ |
|---|---|---|---|
| 1 | 72 | 7 | 2 |
| 2 | 44 | 9 | 2 |
| 3 | 212 | 5 | 1 |
| 4 | 1004 | 47 | 4 |
| 5 | 126 | 18 | 2 |
| 6 | 106 | 5 | 1 |
| 7 | 424 | 33 | 0 |
| 8 | 538 | 33 | 0 |
| 9 | 117 | 10 | 2 |
| 10 | 76 | 6 | 0 |

# Appendix 3

*Appendix 3: Results from PCA plates on the 14th of February*

| 14-feb Loins | PCA | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ |
|---|---|---|---|---|
| 1 | | 5 | 1 | 0 |
| 2 | | 11 | 2 | 1 |
| 3 | | 22 | 1 | 0 |
| 4 | | 27 | 40 | 1 |
| 5 | | 57 | 8 | 0 |
| 6 | | 38 | 0 | 10 |
| 7 | | 51 | 5 | 1 |
| 8 | | 41 | 2 | 1 |
| 9 | | 15 | 0 | 0 |
| 10 | | 3 | 0 | 0 |

| Necks | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ |
|---|---|---|---|
| 1 | 520 | 31 | 2 |
| 2 | 86 | 3 | 1 |
| 3 | 680 | 60 | 5 |
| 4 | 36 | 4 | 0 |
| 5 | 42 | 7 | 1 |
| 6 | 51 | 2 | 0 |
| 7 | 102 | 9 | 1 |
| 8 | 37 | 7 | 0 |
| 9 | 63 | 14 | 1 |
| 10 | 106 | 9 | 4 |

# Appendix 4

*Appendix 4: Results from VRBG plates and oxidase tests*

| Loins | | | |
|---|---|---|---|
| Date | $10^{-1}$ | $10^{-2}$ | Oxidase |
| 31-jan | 1 | | Negative |

| Necks | | | |
|---|---|---|---|
| Date | $10^{-1}$ | $10^{-2}$ | Oxidase |
| 7-feb | 1 | | Negative |

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