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Final report

Improved cleaning and disinfection at meat premises – reducing spread of spoilage-, pathogenic- and antimicrobial resistant bacteria

Introduction

The project (abbreviated CleanDis) was initiated to investigate and address critical challenges related to Cleaning and Disinfection (C&D) procedures in meat production facilities, with a particular emphasis on bacterial biofilms. Biofilms are structured communities of bacteria that attach to surfaces and become highly resistant to disinfectants and environmental stress. This makes them difficult to remove and allows pathogenic, spoilage, and antibiotic-resistant bacteria to survive and potentially contaminate food products. Biofilms are often found at critical control points, where cleaning is difficult and bacterial survival poses a significant risk. In Sweden, as in other EU countries, food producers are responsible for ensuring hygienic production environments that meet food safety regulations. C&D is a central part of good manufacturing practice, but currently available methods are often based on standardized laboratory tests that do not fully reflect the conditions in real meat processing facilities. For instance, disinfectants are tested on smooth stainless-steel surfaces in controlled environments, while actual production areas may include a variety of materials with wear, scratches, and unevenness that allow bacteria to persist.

The guidance provided by suppliers of C&D products is typically based on standardized laboratory conditions, making it difficult for food producers to evaluate whether the products are truly effective in their specific environment. There are also an uncertainty surrounding the methods used to verify the efficiency of C&D. Commonly used sampling techniques, such as ATP bioluminescence, contact plates, dip slides, and swabbing, can produce inconsistent results depending on surface type, bacterial species, and sampling time. Even after C&D, pathogens and spoilage bacteria such as *Listeria monocytogenes*, *Pseudomonas* spp., and *Leuconostoc* spp. can still be detected on food contact surfaces (García-Sánchez et al., 2017). Some bacteria, for example *L. monocytogenes,* may survive for years in production facilities (Fagerlund et al. 2016). Outbreaks of listeriosis and campylobacteriosis in Sweden, linked to contaminated meat products and insufficiently cleaned transport crates, further highlight the need for more effective C&D strategies (Dzieciolowski et al. 2022).

The CleanDis project was developed to address a set of defined needs in the Swedish meat industry: (i) to map the presence of spoilage-, pathogenic-, and antibiotic-resistant bacteria—including those in biofilms—at meat premises; (ii) to evaluate the effectiveness of current C&D routines under practical, real-world conditions; and (iii) to assess the performance and reliability of the sampling and analytical methods used to monitor cleaning outcomes.

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Materials and methods

The CleanDis project was structured into four interlinked Work Packages (WPs). In brief, WP1 consisted of an interview-based study involving eight slaughterhouses, but also an additional study conducted in collaboration with the SLF project O-18-20-158. WP2 was a field study in which repeated bacteriological sampling was conducted at two selected slaughterhouses. Bacterial strains isolated during WP2 were subsequently used in the laboratory experiments conducted in WP4. WP3 was another field-based investigation carried out at the same two slaughterhouses as WP2, but during separate visits.

WP 1: Common C&D practices and perceived challenges

The objective of this study was to describe the most common C&D practices, identify perceived challenges, and determine critical control points at Swedish meat premises. Eight slaughterhouses were included, four red meat and four poultry facilities, selected to represent different production capacities. Together, these slaughterhouses accounted for approximately 30% of red meat and over 90% of poultry slaughter in Sweden. Data were collected through structured interviews with quality assurance managers of C&D routines and reviews of environmental monitoring procedures. Quantitative and qualitative information was documented on, for example, types of C&D agents used, contact times, application temperatures, methods of application, and the sampling strategies used to verify cleaning efficacy. To understand broader patterns and industry-wide challenges, a thematic analysis of interview transcripts was conducted, highlighting recurring issues and knowledge gaps in hygiene management.

In addition, another objective was to assess how effectively applied C&D routines eliminate/inactivate pathogenic bacteria, here exemplified by *Campylobacter* spp. and *L. monocytogenes*. One red meat and one poultry slaughterhouse were included, and we compared critical food- and non-food contact surfaces sampled before and after C&D. The 484 samples (half of which were collected before and after C&D, respectively) were analysed for *Campylobacter* spp. and *L. monocytogenes* using standard culture methods. This part of the study was performed in collaboration with the SLF project O-18-20-158.

WP2: Comparisons of bacterial detection methods

The objective of this study was to compare the efficiency of different detection methods for identifying spoilage, pathogenic, and antibiotic-resistant bacteria in meat premises, with a focus on critical control points. The study was conducted at one red meat and one poultry slaughterhouse in Sweden. Each facility was visited six times before and six times after C&D, resulting in a total of 626 surface samples collected from food contact and non-food contact areas such as drains, cutting tools, and conveyor belts. Three different sampling methods were used: 1. Swabbing and plating for total aerobic (bacteria) count and *Enterobacterales*, 2. Dipslides for total aerobic count, and 3. ATP-bioluminescence for detection of organic residues (not only bacteria). Sampling was performed according to standard hygiene protocols. The results were evaluated against predefined cleanliness thresholds derived from literature and supplier recommendations. Diagnostic performance (sensitivity, specificity, accuracy, and Cohen's kappa) of dipslide and ATP methods was assessed using swabbing and plating for TAB as the reference method.

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WP 3: Biofilm sequencing

The objective of this study was to determine which bacterial species are present in biofilms at meat premises and to assess whether the species composition differs across surface types and over time. To identify critical control points where biofilms may persist after C&D, ATP-bioluminescence data were collected from various critical points at one red meat and one poultry slaughterhouse at four different time points. Surface areas with consistently high ATP levels were selected for further microbiological analyses. At each of the four sampling occasions, approximately 20 surface sites per facility were swabbed after C&D using DNA-stabilizing swabs, resulting in a total of 160 samples. The samples were analysed using 16S rRNA gene sequencing at the Science for Life Laboratory, Solna. Bioinformatic processing and analyses were conducted at the i-Lab platform, Linköping University. Note that these analyses are ongoing at the time of reporting and preliminary results are therefore presented in the Result section.

WP 4: Efficient C&D, with focus on biofilm

The objective of this laboratory study was to compare the reduction of *L. monocytogenes* and *Campylobacter* in biofilms on conveyor belts with use of different combinations and concentrations of C&D agents commonly used in slaughterhouses with adjacent meat production facilities. To achieve this, the efficacy of selected C&D agents was evaluated against biofilms formed by different strains of *Listeria monocytogenes* and *Campylobacter* spp. on conveyor belt materials used in slaughterhouses. All strains included in this WP originated from WP 2. Biofilms were built on conveyor belts under controlled laboratory conditions designed to mimic meat processing environments, for example by using used conveyor belts from the included slaughterhouses. The tested C&D agents reflected those in use at a large Swedish poultry and red meat slaughterhouse and included both conventional commercial products and novel alternatives, such as enzymatic cleaners and electrolysed water. Three different cleaning agents were tested, followed by different combinations of the cleaning agents with four disinfecting agents. The different combinations of C&D agents were applied at varying concentrations and in either foam or liquid form. Bacterial reductions were measured in log CFU per coupon after treatment with different combinations of the C&D agents. Adequate statistical tests have been performed, and interpretations of the results are currently ongoing.

Results

WP 1: Common C&D practices and perceived challenges

Daily C&D was performed in all slaughterhouses and nine (out of twelve) hired external cleaning companies. Most slaughterhouses used alkaline detergents with or without chlorine, applied using low-pressure systems. Acidic cleaning agents were alternated at 90% of sites. Disinfectants, also mostly chlorine-based, were used and none of the facilities used quaternary ammonium compounds. Quality assurance managers expressed difficulties in determining C&D efficacy, identified several surfaces as difficult to clean and noted reliance on externally provided hygiene thresholds. Several Quality assurance managers also noted that surfaces were often still wet in the morning prior to production, indicating insufficient drying time. Environmental hygiene was monitored using dipslides (80%) and ATP-bioluminescence (70%), with 50% of slaughterhouses using both. Only one facility used swabbing and total aerobic counts. Almost all facilities (90%) included *Listeria* spp. monitoring in floor drains

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and half had detected *Listeria* at some point. Thresholds for microbial contamination were typically based on guidance from external laboratories or suppliers rather than internal risk assessment. Surfaces frequently described as hard to clean included cutting tools, conveyor belts, machine interiors, and dehairing scrapers. Many of these had design or material features (e.g. plastic with scratches) that made effective cleaning more difficult.

Four main themes emerged from the qualitative interviews:

1. Knowledge gaps in microbial composition – Qualitative assurance managers expressed interest in better understanding which bacteria are present and how to effectively target them.
2. Efficacy of C&D and need for scientific support – There was uncertainty about whether current C&D procedures were optimal, and a desire for science-based guidelines.
3. Staff competence and management – Frequent contractor changes and a lack of skilled personnel were highlighted as challenges.
4. Challenges related to production and competitiveness– Competitive concerns hindered collaboration between slaughterhouses, even though managers saw the value of experience exchange.

In the additional study, it was shown that *Campylobacter* spp. were isolated from 13% to 16% of samples (n=242) before C&D in the red meat and poultry slaughterhouse, respectively. *Listeria monocytogenes* was isolated before C&D in 13% and 5.2% of samples in the red meat and poultry slaughterhouse, respectively. *C. jejuni* was detected on multiple surfaces and *L. monocytogenes* showed potential persistence in one slaughterhouse. It was shown that the C&D procedures efficiently removed/inactivated *Campylobacter* spp. and *L. monocytogenes* from surfaces in the included slaughterhouses. Only one *L. monocytogenes* was isolated in the samples (n=242) collected after C&D.

WP2: Comparisons of analytical methods

In total, 626 surface samples were collected from a red meat and a poultry slaughterhouse, both before and after C&D. For the majority of samples, total aerobic count was lower after than before C&D and bacteria belonging to order *Enterobacterales* were mainly detected before C&D, indicating C&D efficacy. Greater reductions in mean total aerobic count were observed in processing areas (2.2 and 2.8 log CFU/100 cm2 in red meat and poultry slaughterhouses, respectively) than in slaughter areas (1.3 log CFU/100 cm2 in both slaughterhouses).

Approximately half of all samples were assessed as non acceptably clean (52% for red meat and 46% for poultry slaughterhouse) according to previously published thresholds. Critical food contact surfaces that were insufficiently cleaned and disinfected were, for example, plucking fingers, shackles, and a post-dehairing table. These are surfaces that often are difficult to clean and/or with worn surfaces. C&D of drains and floors were also inadequate. Overall, *Enterobacterales* were detected on 7% (red meat) and 25% (poultry) of surfaces after C&D. The ATP-bioluminescence method showed low specificity compared with the reference (total aerobic count) in both the red meat (0.30) and poultry slaughterhouses (0.64). The sensitivity of dipslides was low (0.26) in the red meat slaughterhouse compared with total aerobic count. A combination of ATP-bioluminescence and dipslides could provide more accurate estimates of C&D efficacy. It was also that the slaughterhouses included in this study

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put less effort into monitoring the cleanliness of food contact surfaces in slaughter areas compared with non-food contact surfaces.

WP 3: Biofilm sequencing

A total of 111 surface swab samples collected from four slaughterhouses were analysed using 16S rRNA gene sequencing. Sequencing revealed 615 bacterial genera across all samples, although most were found only sporadically. The most commonly detected genera were *Psychrobacter* and *Acinetobacter*, both psychrophilic and frequently associated with meat and meat products. These dominated the microbial communities, with an average relative abundance of 19%. Seven bacterial genera known to contribute to meat spoilage were consistently present, including *Pseudomonas* and *Brochothrix*, and together accounted for approximately 10% of the average abundance. Five genera associated with foodborne illness were also detected: *Yersinia*, *Staphylococcus*, *Listeria*, *Klebsiella*, and *Campylobacter*. However, these potential pathogens occurred at low levels, representing only about 0.1% of the average microbial abundance.

Ongoing analyses aim to compare microbial community structures between food contact and non-food contact surfaces, with particular focus on floor drains, recognized critical control points for biofilm formation, and to assess how bacterial biofilm communities evolve over time.

WP 4: Efficient C&D, with focus on biofilm

Overall, the highest mean reductions ranged from 1.7-2.2 log CFU/mL and the lowest from 0-0.2 log CFU/mL, that is per tested combination of different cleaning and disinfection agents per biofilm coupon. No large difference between different treatments were observed and high bacterial loads were still present on the coupons after the treatments. The highest reduction was observed when an alcohol-based disinfectant was combined with a high-concentration chlorinated alkaline cleaning agent. The lowest efficacy was seen when using cleaning agents alone or acids at low concentration. When only cleaning agents were applied, higher concentrations of both chlorinated alkaline and acidic agents resulted in more effective reduction of *L. monocytogenes*, with approximately 0.5 log greater reduction than at lower concentrations. In general, there was no improved bacterial reduction when enzymes replaced acidic or chlorinated alkaline cleaning. For both bacteria., the combination of high-concentration chlorinated alkaline agent and alcohol achieved the highest reduction, 1.7-2.2 log CFU. In contrast, treatment with electrolyzed water resulted in low reduction, around 0.7 log. The results show that high concentration of the chemicals (chlorinated alkaline, acidic and enzymatic agents) produce the highest reduction of biofilm. The final results are currently being evaluated.

Discussion

The CleanDis project revealed significant variation and challenges in C&D practices across Swedish slaughterhouses. Most slaughterhouses included in the epidemiological study outsourced daily C&D due to limited internal capacity or expertise, and also highlighted a need for better, science-based C&D guidelines. Only half of the slaughterhouses used thresholds based on internal risk assessments, while others relied on external recommendations, risking misaligned hygiene controls. Nine out of ten included slaughterhouses monitored *Listeria* spp., as required by EU legislation (European

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Commission, 2005), and most had implemented control measures. This may lead to food safety challenges as it has been shown that, for example, *L. monocytogenes* can persist for many years in a food processing plant drain (Fagerlund et al., 2016). Quality assurance managers expressed a need for in-situ, science-based evaluations of C&D procedures and better collaboration with researchers. A lack of knowledge transfer and limited exchange of experiences were also noted barriers to improving hygiene routines (Braun et al. 2011).

The project confirms that visually clean surfaces do not always equate to microbiologically clean ones, for example in the poultry slaughterhouse where irregular surfaces like plucking fingers and shackles remained contaminated after C&D, supporting findings from García-Sánchez et al. (2017). Contamination was generally higher in the slaughter area compared to the processing area. For example, post-dehairing tables in the red meat plant showed high total aerobic counts after C&D. Drains and scratched surfaces like organ conveyor belts also retained high bacterial loads, potentially allowing for the development of resident microbiota that could spread to food-contact surfaces. It was also shown that the C&D procedures used at the two included slaughterhouses efficiently remove the pathogenic bacteria *Campylobacter* spp. and *L. monocytogenes*, but also indicate that deficiencies in slaughter hygiene pose a risk of cross-contamination of meat (Moazzami et al. 2025).

It was shown that a majority of include slaughterhouses relied on dipslides and ATP-bioluminescence to monitor efficacy of C&D (Moazzami et al. 2023). These methods were therefore evaluated in the project and showed varying reliability. Both methods are user-friendly but have limitations: dipslides lack sensitivity, and ATP tests detect non-bacterial residues such as fat or blood. The laboratory study showed that dipslides had low sensitivity, especially in the red meat plant, likely due to poor access to crevices, compared with swabbing and plating of total aerobic count. ATP-bioluminescence, though fast, often gave false positives due to detection of organic debris like blood or fat. Combining dipslides and ATP will improve hygiene monitoring. It should also be mentioned that there are no standardized thresholds for when a surface is considered clean. Each food business operator should therefore select their own threshold based on trends over time. Findings from CleanDis emphasis the need for harmonized cleanliness thresholds and risk-based monitoring in slaughterhouses.

Sequencing offered a detailed picture of microbiota composition at the selected critical points. Although spoilage organisms were common, the presence of potentially pathogenic genera, even in low abundance, indicates a latent risk to food safety if biofilms are not sufficiently eliminated. Overall, the meat processing plant microbiota differed between different sampling points, which might be affected by temperature, slaughter process and effect of C&D with a greater presence of psychrotrophic taxa in secondary processing rooms because of their lower temperatures (Botta et al 2020).The observed microbial diversity highlights the need to tailor C&D efforts to the specific microbiota at each slaughterhouse.

As it was shown that conveyor belts were considered particular difficult to clean, often due to material damage and complex equipment, it was decided to use worn conveyor belts in the laboratory study investigating the effect of different C&D strategies for eliminating biofilms. It was demonstrated that C&D efficacy varies significantly depending on the choice, concentration, and combination of agents. Acid and enzymatic treatments showed the best performance, but even the most effective combinations only achieved reductions of 0.5–2 $\log_{10}$ CFU. This is lower than recommended in the EN 1276 regulation stating that a 5 log reduction is needed for a chemical claiming to have bactericidal effect. The results obtained

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support the notion that biofilms are inherently resistant to conventional C&D protocols, especially under real-world conditions involving worn surfaces and complex equipment. *Campylobacter* biofilms appeared easier to reduce than *Listeria*, though differences in starting bacterial loads may explain this. The results indicate that current real-world cleaning practices may be insufficient for eliminating biofilms under certain conditions.

Conclusions

CleanDis highlights key challenges in C&D, and environmental monitoring practices in Swedish red meat and poultry slaughterhouses. Despite daily C&D routines, variations in procedures, chemical usage, and monitoring strategies were evident. Knowledge gaps regarding microbial composition on surfaces, the efficacy of C&D protocols, and the lack of science-based guidelines were identified as significant concerns among quality assurance managers. Addressing this requires stronger partnerships between industry and the scientific community to develop practical, evidence-based guidelines. Enhanced training, better hygienic design considerations, and risk-based monitoring thresholds could further improve hygiene standards.

The project also highlighted challenges how to assess surface cleanliness after C&D. This include the risk of missing bacteria when using only dipslides and the difficulty of interpreting the ATP-bioluminescence results, as this method does not only measure the microbial load. An alternative would be to combine the two methods, and to use swabbing and plating (total aerobic count) to verify results. There is also difficulty in interpreting the results of monitoring operations, due to the absence of commonly agreed guidelines on when a surface is sufficiently cleaned. It is concerning that the slaughterhouses included in this study put less effort into monitoring the cleanliness of food contact surfaces in slaughter areas, even though these surfaces may constitute the greatest risk to meat cross-contamination, considering direct contact with the product. This may constitute a risk for the spread of bacterial foodborne pathogens.

It was shown that biofilms in slaughterhouse environments are dominated by bacteria naturally associated with meat and processing environments, but also contain spoilage organisms and low levels of potential pathogens. The presence of these bacteria, even in small amounts, reinforces the need for continuous hygiene procedures, especially at sites where biofilms can persist. Monitoring sites where biofilms can persist might provide an important baseline for assessing microbiological risks. CleanDis also confirms that biofilm formed by foodborne pathogens can be difficult to eliminate using commonly available C&D agents. Although some treatment combinations yielded greater bacterial reductions, no method fully removed biofilms to levels recommended by international standards. Differences in efficacy between treatments were more influenced by concentration and combination rather than the specific type of agent.

Relevance and recommendations

The CleanDis project has contributed with knowledge that is important when developing evidence-based guidelines targeting practical hygiene strategies adapted to real-world meat processing facilities. By highlighting the risk of persistent presence of spoilage- and pathogenic bacteria in slaughterhouse environments, despite regular C&D procedures, the project underscores the importance of effective C&D, followed by regular environmental monitoring of indicator bacteria. This is particularly important considering the findings of *L.*

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monocytogenes and *Campylobacter* spp. at critical control points, including on surfaces that appear clean.

There is a clear demand for science-based guidelines that can be used by the slaughterhouses. This includes a better collaboration between industry and the research community to develop C&D strategies that are effective in complex, real-life environments. There is also a demand from the Quality assurance managers at the slaughterhouses to increase knowledge about hygiene and C&D routines at the slaughterhouses, as a way of increasing quality and safety of produced products. C&D routines often rely on supplier guidelines, which do not take specific conditions at the various slaughterhouses into account, including worn, scratched or irregular surfaces.

It is also needed to develop harmonised hygiene thresholds of what is considered a clean surface. Without clear, science-based criteria for what constitutes a “clean” surface, food business operators risk misinterpreting results and overlooking contamination. Slaughterhouses also need support on how to perform effective environmental monitoring to assess hygiene criteria. In this study, 16S rRNA sequencing was performed. This can offer a deeper understanding of microbial communities in food processing environments and could help prevent biofilm development and persistence over time. Integrating such tools periodically into monitoring routines could support the development of more targeted and risk-based hygiene protocols.

Overall, CleanDis has met the stated objectives and thereby contributed to strengthening the scientific foundation for C&D strategies and environmental monitoring in Swedish slaughterhouses. The insights gained can support better hygiene outcomes, reduced contamination risks, and ultimately safer meat products of high quality.

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Result dissemination:

Scientific publications, *published*	*Moazzami M, Bergenkvist E, Boqvist S, Frosth, Langsrud S, Møretrø T, Vågsholm I, Hansson I. 2023. Assessment of ATP-Bioluminescence and Dipslide Sampling to Determine the Efficacy of Slaughterhouse Cleaning and Disinfection Compared with Total Aerobic and* Enterobacterales *Counts. Journal of Food Protection. 10:86. https://doi.org/10.1016/j.jfp.2023.100155*
	Moazzami M, Bergenkvist E, Boqvist S, Frosth, Langsrud S, Møretrø T, Vågsholm I, Hansson I. 2024. Occurrence of Campylobacter, Listeria monocytogenes, and extended-spectrum beta-lactamase Escherichia coli (ESBL E. coli) in slaughterhouses before and after cleaning and disinfection. Food Microbiology. 125:104639 doi.org/10.1016/j.fm.2024.104639.
Scientific publications, *submitted*	*Moazzami M, Gröndal H, Hansson I, Boqvist S. Challenges in cleaning and disinfection and environmental monitoring in Swedish slaughterhouses.*
Scientific publications, *manuscript*	*Moazzami M, Nasirzadeh L, Langsrud S, Møretrø T, Hansson I, Boqvist S. Listeria monocytogenes and Campylobacter spp. biofilm removal from conveyor belts using different commercial cleaning and disinfecting agents (to be submitted in September 2025)*
	Moazzami M, Nasirzadeh L, Hansson I, Boqvist S. Temporal and Surface-Dependent Variations in Biofilm Microbiota at Meat Processing Facilities. (to be submitted in October 2025)
Conference publications/ presentations	*Moazzami M, Bergenkvist E, Boqvist S, Frosth S, Langsrud S, Møretrø T, Vågsholm I, Hansson I. Occurrence of Campylobacter in slaughterhouses before and after cleaning and disinfection. CHRO Perth, October 7-9, 2024 (oral presentation)*
	Moazzami M. Cleaning and disinfection in the slaughterhouse. Comparison of sampling methods by bacterial enumeration. A way to prevent transmission of foodborne pathogenic and spoilage bacteria. Swedish Veterinary Congress. Nov 2022. (0ral presentation)
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Other publications, *media etc.* 2023,	*Industrin saknar generella riktlinjer för att rengöra ytor, Nov 29, 2023. Veterinärmagazinet (*Industrin saknar generella riktlinjer för att rengöra ytor - VeterinärMagazinet)
	*Rengöring och desinfektion kan förbättras på svenska slakterier. 2023, Nyhet SLU hemsida, samt press release (*Rengöring och desinfektion kan förbättras på svenska slakterier | Medarbetarwebben)
	Slakterierna är inte rena nog. Nr 8, Dec 2023
	*Bristfälliga riktlinjer för rengöring och desinfektion på slakterier, Svensk veterinärtidning, Nr 1, Jan, 2024 (*SVT2401_lores.pdf)
	Så får vi rent slakteri, Feb 2024. Kött & Chark
	*Rengöring och desinfektion. Dec 2023, Jordbrukaren (*Rengöring och desinfektion | Jordbrukaren*)*
Oral communication, *to sector, students etc.*	*2022. Meetings with all six slaughterhouses that participated in the various field studies within project to disseminate results.*
Student theses	*Student: John Johansson, Supervisor: Madeleine Moazzami, 2024. Design and validation for Clean-in-Place (CIP) in a food process, Master's thesis in Food Science.* johansson-j-250302.pdf
Other including Patents	*Doctoral thesis: Madeleine Moazzami, Supervisor: Sofia Boqvist, Ingrid Hansson, Foodborne bacteria in slaughterhouses: with focus on cleaning and disinfection, 2023. (*https://publications.slu.se/?file=publ/show&id=126461&lang=se)

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