Date: 2022-06-30

Final report

Measures to reduce the proportion of chickens with *Campylobacter* in Sweden

Project number: O-18-20-158

Introduction

Campylobacter spp. is the most reported bacterial cause of gastrointestinal disease in humans in Europe and many other parts of the world. Data available from several studies show a significant economic impact through loss of working time and disease burden as a result of infection with *Campylobacter*. In 2017, Swedish chicken received very negative attention in media in connection with an increased number of human cases of campylobacteriosis. This type of attention led to a reduction in the consumption of Swedish chicken. Increased knowledge of how *Campylobacter* is introduced and spread in the flock and how to avoid *Campylobacter* contaminating chickens during slaughter and further processing is of the utmost importance to reduce the proportion of chicken products with *Campylobacter* at consumer level. The project outcome was to provide recommendations for measures to prevent *Campylobacter* from colonizing chickens at farm level and how to avoid contamination at slaughter.

Materials and methods

Quantitative analysis *Campylobacter*

The quantitative analysis was performed according to ISO 10272 part 2 (ISO, 2017). Briefly, sock samples or 10 g of other types matrix were homogenized in a stomacher for 1 min at 240 rpm. A 10-fold serial dilution in 0.1% (v/v) peptone water (Dilucups, LabRobot Products AB) was prepared and 0.1 ml each from each dilution were plated onto a modified Charcoal Cefoperozone Deoxycholate agar (mCCDA). The plates were incubated at 41.5°C ± 0.5°C for 44 ± 4 h in a microaerobic atmosphere by the use of Campygen in anaerobic jars. After incubation, colonies characteristic of *C. jejuni* were quantified and the number was expressed as $\log_{10}$ CFU/g. The detection limit was 1.0 CFU/g or 1.0 CFU/ml. All *Campylobacter* isolates identified were stored in Brain Heart Infusion (BHI) broth (CM1135; Oxoid) with 15% glycerol at −70 °C.

Qualitative analysis *Campylobacter*

All samples were cultured for *Campylobacter* according ISO10272-1 (2017). Qualitative analysis of faces samples was analysed by direct culture on mCCDA and incubated at 41.5 °C in a microaerophilic atmosphere for 44 h. The sock and swab samples were analysed by enrichment in 90 mL Bolton Broth and incubated at 37.0°C for 4–6 h in a microaerobic atmosphere, followed by incubation at 41.5°C for 44 h. Two loops of enriched culture (approximately 20 µL) were cultured on mCCDA plates which were incubated at 41.5°C for 48 h in a microaerobic atmosphere. All *Campylobacter* isolates identified were stored in Brain Heart Infusion (BHI) broth (CM1135; Oxoid) with 15% glycerol at −70 °C.

Analysis of total number aerobic bacteria

The total number of live, aerobic bacteria were analysed according to NMKL-method 86. In brief, 90 mL buffered peptone water (BPW) were added to the swab/meat sample and stomached 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 plate count agar (PCA) in a Petri dish (9 cm diameter). After agar solidification, the plates were incubated at 30.0°C for 72±7 hours. Plates with 25-250 colonies were selected for quantification, since these are considered to give the most accurate microbiological results.

Analysis by dipslides (Envirocheck® Contact TVC Merck KGaA, Darmstadt, Germany) were performed by firmly pressing both sides of the dipslide onto the surface. The dipslides were thereafter incubated in

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upright position at 37 ± 1 °C and was checked for bacterial growth after for 48 ± 4 h. Number of colonies were counted on both sides of the slide. The colony count on each agar side was calculated into CFU per cm^2 by: CFU (actual count on both sides) / 19 cm^2 = CFU/cm^2.

Quantitative analysis of *Enterobacterales*

Analysis of bacteria belonging to order *Enterobacterales* was performed according to NMKL 144 (3rd Ed., 2005). 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 violet red bile glucose agar (VRBG) in a Petri dish and left to solidify, and then an overlay of 5 mL VRBG was added and the plates were incubated at 37°C for 24 h. The numbers of suspected bacteria belonging to the *Enterobacterales* were counted on plates with 15-150 colonies. Five colonies preliminarily identified as *Enterobacterales* were cultured on blood agar and incubated at 37°C for 24 ± 2 h. Presence of bacteria belonging to the *Enterobacterales* was confirmed by oxidase test and/or MALDI-TOF MS. The detection limit was log 1.0 CFU/mL.

Whole-Genome Sequencing

Genomic DNA was extracted from *Campylobacter* isolates subcultured twice from single colonies on horse blood agar plates for 48 h at 41.5 °C in a micraerobic atmosphere, using the EZ1 DNA Tissue Kit and the bacterial protocol on an EZ1 Advanced XL. The elution volume used was 100 µL and the DNA concentration was measured using the Qubit ds DNA Broad Range assay kit on a Qubit® 2.0 Fluorometer. Sample libraries were prepared using the Nextera XT DNA Library Preparation Kit. Whole-genome sequencing was performed on an Illumina NextSeq 500 system with 2 × 150 bp paired-end reads using the NextSeq 500 Mid Output kit V2.5. The resulting sequences were trimmed and filtered using fastp and *de novo* assembled using the SPAdes or SKESA assembler. For core genome MLST (cgMLST) analysis, Ridom SeqSphere+ or the chewBBACA pipeline, which performs scheme creation and allele calls on complete and draft genomes, was used. The cgMLST results based on core genome allelic distances were visualized using Ridom SeqSphere+ or GrapeTree.

Adenosine triphosphate levels (ATP) - bioluminescence (measures levels of cellular materials)

The area was swabbed with light pressure by overlapping horizontal and vertical strokes and at the same time the swab was rotated over its own axis according to the manufacturer's instructions. The ATP level was measured by placing the swab into the ATP monitoring device (Clean-Trace LM1, 3M Health Care, St. Paul, USA). Results were reported in approximately 8 s recorded in relative light units (RLU) in the system device range of 0 to 1000000 (0 to 6 log RLU).

Results

The first aim of the project was to identify risk factors for *Campylobacter* in Swedish broiler flocks, particularly with regard to broiler house environment, design of buildings and farm management practices

An in-depth analysis was performed on Swedish broiler producers that had delivered chickens with *Campylobacter* to slaughter over several years, in order to identify possible transmission routes and formulate effective measures to prevent chickens being colonized with *Campylobacter*. A total of 626 samples were collected at farm level and *Campylobacter* were isolated from 133 (23.2%). All *C. jejuni* and *C. coli* isolated from these samples were whole-genome sequenced, together with isolates from the corresponding cecum samples at slaughter (n=256). cgMLST analysis, using schemes consisting 1140 and 529 genes *C. jejuni* and *C. coli*, respectively, revealed that nearby cattle, contaminated drinking water, water ponds, transport crates, and parent flocks were potential reservoirs of *Campylobacter*. A novel feature compared with previous studies is that measures were implemented and tested during the work. These contributed to a nationwide decrease in Campylobacter-positive flocks from 15.4% in 2016 (before the study) to 4.6% 2020 and 5.3% in 2021, which is the lowest ever rate in Sweden. To conclude, there are different sources and routes of Campylobacter transmission to chickens at different broiler producers,

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and individual measures must be taken at each producer to prevent *Campylobacter* colonization of chickens (Frosth et al 2020).

Good hygiene together with other biosecurity measures is of importance for prevention and control of diseases. All broiler houses in Sweden are cleaned and disinfected between the flocks, with different cleaning and disinfection (C&D) protocols in use and the execution of them varies. There is no evaluation system in place to enable a systematic assessment of the protocols being used. A study were performed with the aim in (a) obtaining information regarding the effectiveness and (b) carry out the comparison of different C&D protocols on broiler farms. Furthermore, since there is no guideline regarding the desirable reduction of bacterial load after C&D, the obtained results of the bacterial testing should (c) provide a useful information on the topic, which should facilitate development of such a guideline. C&D procedures on broiler farms were examined by testing of different sampling points using double-sided dipslides. Initially, 25 randomly chosen broiler farms were assigned to participate in the study. Finally, 1540 samples were analysed from 18 farms that completed the project according to the plan. The results show a substantial variation in bacterial counts between different farms ($P<0.001$). However, there was no difference in bacterial counts between different sampling points in the broiler house (Figure 1). Neither length of downtime nor time between cleaning and disinfection stage correlates with bacterial counts. Neither the year of build nor the size of the broiler house seem to be associated with bacterial counts. All participating farms except one have used detergent during the cleaning. Although there was no statistical difference in bacterial load associated with use of detergent in our statistical model, it is worth to mention that the farm who did not use it, had the highest average bacterial load at all sampling occasions. There were no significant differences in bacterial loads between different disinfection products (Dzieciolowski et al in manuscript).

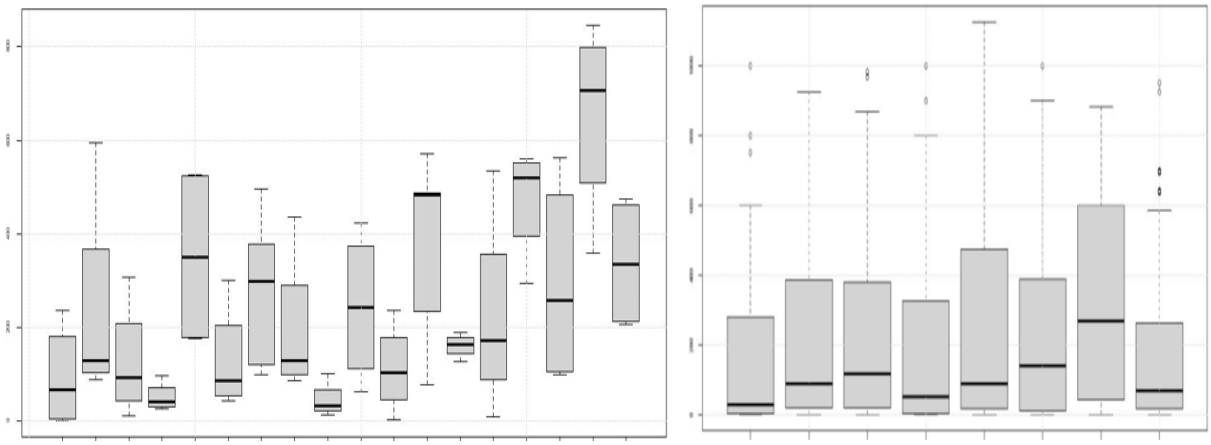

Figure 1. Distribution of bacterial counts in dip-slides samples taken on different farms (A) and sampling points (B).

The second aim was to evaluate and validate the sampling methods of *Campylobacter* in water and the impact of different cleaning system and detergent of the water pipes and in the long run how to avoid survival of *Campylobacter* in the biofilms in the water pipes.

Bacteria and microbes can grow on the inside of water pipes, which appears in the form of a thin and sticky coating - biofilms. Biofilms enable microorganisms to survive in environments where they normally do not survive. Before taking samples from drinking water, flushing with water and air should be performed. This increasing and decreasing the pressure of the water in the pipes, facilitate the removal of the biofilm in the pipes, and increase the possibility to isolate *Campylobacter* from water (Frosth et al 2020). One of our hypothesis is that *Campylobacter* can survive in the biofilms due to inadequate cleaning and disinfection of the water pipes, and may pose a risk to the following chicken flocks getting colonised with *Campylobacter*. A study was performed with the purpose to produce biofilm and investigate how biofilm was affected by six different disinfectants that are often used in farms, slaughterhouses and food premises. Biofilm was produced by *C. jejuni* in mixed culture with six other bacterial species in a static

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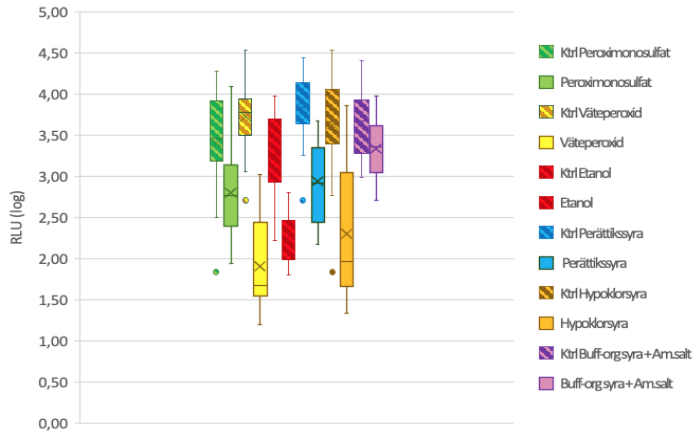
model by microtiter plates and a dynamic model with pieces of plastic water pipes previously used in broiler houses. The disinfectants tested were hypochlorous acid, hydrogen peroxide, peracetic acid, 70% ethanol, buffered acids and peroxymonosulphate. A total of 3264 microtiter wells and 765 water pipe sections were analyzed in order to make an overall assessment of which disinfectant had the most effect on removing biofilm and *C. jejuni*. All disinfectants reduced the amount of bacteria. The largest mean reduction were found after treatment of hydrogen peroxide both in the microtiter plates and water pipes. The least effect was found after treatment of buffered acids (Figure 2).



	Control (log RLU)	Des inf (log RLU)	Reduction (log RLU)
Peroxymono sulphate	3.41	2.8	0.61
Hydrogene peroxide	3.73	1.91	1.82
70% Ethanol	3.25	2.24	1.01
Peracetic acid	3.83	2.94	0.89
Hypoclorous acid	3.63	2.30	1.33
Buffered acids	3.65	3.34	0.31

Figure 2. Distribution of logarithmic ATP values of relative light units (RLU) in water pipes after treatment in one hour of the various disinfectants and positive control with rinsing of sterile water.

C. jejuni could be not be detected from any of the water pipes pieces after treatment with hydrogen peroxide, hypochlorous acid, and peracetic acid. Less effective at killing *C. jejuni* in biofilm were buffered acids where *C. jejuni* could be detected from 53% of the water pipes after treatment. *C. jejuni* could be detected from 15% and 16%, respectively, of the water pipe pieces treated with peroxymonosulphate and 70% ethanol, respectively (Figure 3).

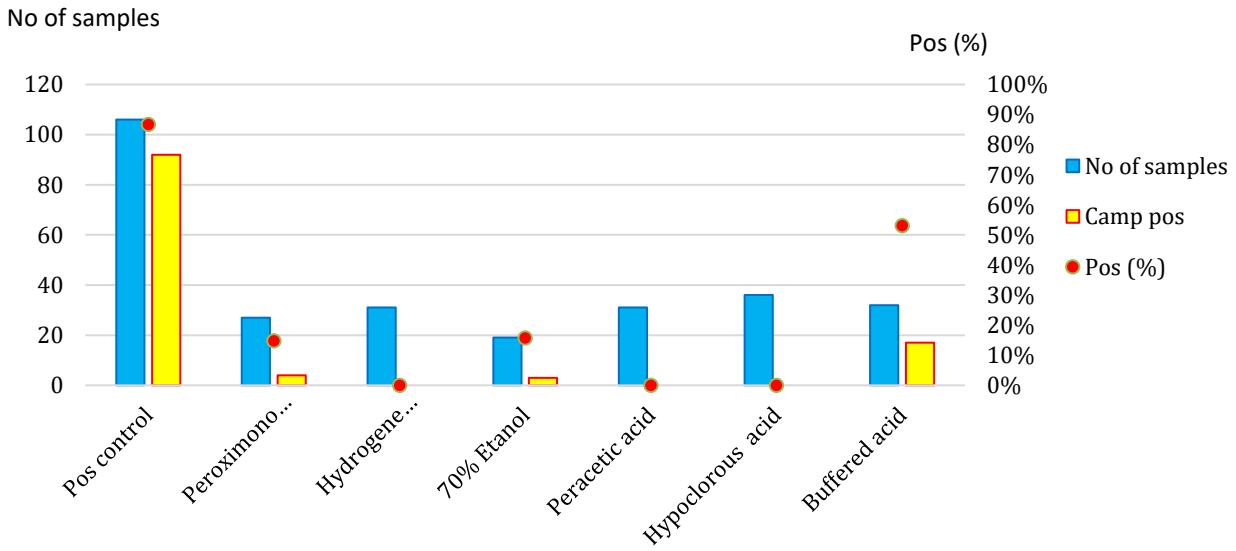


Figure 3. Presence of *Campylobacter jejuni* in biofilm in water pipes after treatment with six different disinfectants and positive controls of water pipes treated by water.

The results indicate that the disinfectant must be chosen based on the specific environment as well as the bacteria it aims to eliminate, since the effectiveness differs between different types of biofilms and the agents present. It was also shown that the effect of mechanical cleaning cannot be replaced by disinfectants alone. However, hydrogen peroxide has the greatest ability to disinfect several different

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environments with different types of biofilms and can serve as a complement to multidisciplinary cleaning routines (Viklund 2021).

The third aim of the project was to evaluate and streamline the cleaning of transport crates to prevent the transfer of *Campylobacter* to other chickens.

Transport crates for poultry can contribute to the spread of *Campylobacter*. A strict cleaning procedure and use of an effective disinfection method for transport equipment are thus important to avoid introduction of *Campylobacter* to chickens, particularly during thinning of flocks. This study evaluated the efficacy of the disinfection procedure currently in use (Trial A) at one of the largest slaughter plants in Sweden and compared the effects with those of other disinfection methods. The evaluation was based on treatment ability to reduce the presence and amount of indicator bacteria *Enterobacterales* and total aerobic bacteria. In four trials, sodium hypochlorite (Trial A), peracetic acid (Trial B), and drying with hot air, without (Trial C), or with sodium hypochlorite (Trial D) for final disinfection, were compared.

The results showed that use of a chemical disinfectant in combination with drying with hot air (Trial D) was the most effective treatment, with an average reduction of 3.4 log for total aerobic bacteria and 3.8 log for *Enterobacterales* (Figure 4*).* Since all crates treated with hot air were dry, transport conditions for the birds also improved, particularly in cold weather. A disadvantage is that this treatment is energy-consuming and would require substantial technical changes to the current cleaning process, increasing operating costs at the slaughter plant. However, considering the contribution of improved cleaning to overall hygiene control within the poultry supply chain and the beneficial effect on animal welfare, the costs may be justified (Dzieciolowski et al. 2022).

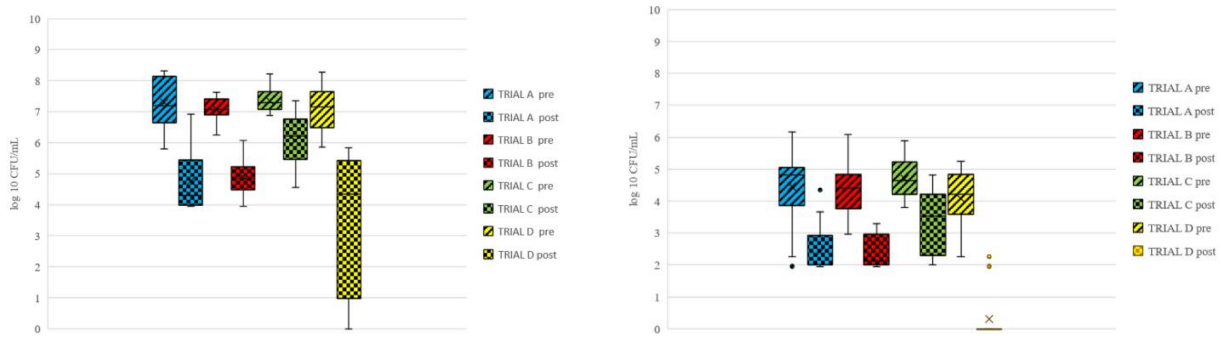

Figure 4. Mean counts of total number of aerobic bacteria and *Enterobacterales* (log CFU/mL) detected on swab samples taken from chicken transport crates before (pre-) and after (post-) cleaning in trials A–D.

The fourth aim of the project was to evaluate measures that can be carried out at slaughter level to reduce the amount of *Campylobacter* on the chickens after slaughter.

In the food industry, surfaces that are in direct contact with food products, processing equipment and other non-food contact surfaces require cleaning followed by disinfection in order to minimize the number of microorganisms and thereby preventing contamination during production. The amount of microorganisms should be reduced to a minimum to minimize the risk of contamination, spread and multiplication of foodborne pathogenic bacteria. Chemicals commonly used for C&D in meat and poultry premises are alkaline- and chlorine compounds (i.e. sodium hypochlorite), acidic compounds (i.e. peracetic acid) and quaternary ammonium compounds. The aim of this study was to enumerate hygiene indicator bacteria on food contact- and non-food contact surfaces in the slaughterhouse before and after C&D and to compare the results of rapid and traditional sampling methods used to evaluate the efficiency of the C&D procedures. The sampling procedure was divided into two areas: slaughter area and adjacent meat processing area (cutting area). Eleven critical control points (CCP:s) in the slaughter area and 10 CCP:s in the meat processing area were subjected to sampling. The same sampling methods were used and the same CCP:s were targeted at each occasion, before (A) and after (B) C&D procedures, which were after the end of the last working shift/before C&D and after C&D/before the start of the morning shift.

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Sampling was performed with three different methods: swabs measuring ATP, dipslide tests and swabbing with sponges or swabs for analyzing total number of aerobic bacteria. The majority of the surfaces were wet at the time of sampling especially after C&D. Some surfaces were visibly dirty even after C&D. Total number of aerobic bacteria could be was enumerated in all A-samples and in 73% of the B-samples. In 46% of the B-samples, the values were above 2.4 log CFU/100 cm² (2.5 CFU/cm²). Bacteria belonging to *Enterobacterales* could be enumerated in 88% of the A-samples with a mean of log 2.4 ± 0.9/100 cm². The mean reduction was 2.1 ± 0.6 CFU/100 cm². *Enterobacteriaceae* could be enumerated in 25% of the B-samples: on plucking fingers, chacle and on the floor and well. ATP was quantified in all A- and B- samples 67% of the B-samples were above the acceptable limit. The highest B-value was found on the plucking fingers (Moazzami et al in manuscript).

One post-harvest preventive measure to reduce *Campylobacter* concentrations on poultry meat is freezing. Survival of different sequence types of *C. jejuni* during freezing and transmission in the kitchen were examined by artificially contamination of broiler fillet before freezing with two different sequence types and concentrations of *C. jejuni* (ST-257 and ST-918). Freezing reduced the concentrations of *C. jejuni* in broiler meat and meat juice, but did not eliminate the presence of all bacteria. The rate of decrease in *Campylobacter* concentrations on frozen chicken broiler fillet was greatest in the first four days of freezing and flattened off thereafter. There was a difference in the ability of *C. jejuni* standard types to withstand the stress of freezing, with ST-918 decreasing to a lesser extent than ST-257, indicating a possible difference in their ability to cause disease. In the kitchen, the meat juice probably poses a greater risk than undercooked core meat, since the juice had similar concentrations of *C. jejuni* to the uncooked meat and the juice can easily spread to other surfaces in the kitchen (Eriksson et al submitted).

The fifth aim of the project was to compare *Campylobacter* isolates from different places in the production chain with the purpose to identify risk factors important for colonisation of broilers and further contamination at slaughter.

Previous studies have not found any evidence for vertical transmission of *Campylobacter,* ie it spreads from the hen to the egg although studying about 60,000 progeny parent breeders that were hatched from eggs coming from *Campylobacter*-positive grandparent flocks. Callicot et al (2006) concluded that if vertical transmission is occurring, it is not a significant source for the contamination of chicken flocks with *Campylobacter* spp. Despite this, we have found the same sequence types of *Campylobacter* from parent flocks and broiler chickens by the use of WGS at several times for different ST, ST-19, ST-48, ST-148, ST-221 and ST-9198. Vertical transmission occur for certain serotypes of salmonella, such as *Salmonella* Typhimurium and *Salmonella* Enteritidis (Timoney et al. 1989), but not *Campylobacter* spp. However, it is not known if *Campylobacter* can be transmitted from parent birds to chicken via contaminated eggshells. The intestines of poultry can be colonized with *Campylobacter* up to log 8 CFU/g (Hansson et al. 2010). Eggshells may be contaminated with *Campylobacter* after being exposed to faeces during laying, which in theory can be transmitted to newly hatched chicks. There is a lack of knowledge of the risk for humans being infected with *Campylobacter* via contaminated eggshells, and if/how often *Campylobacter* can be transmitted from parent birds to newly hatched chicks via contaminated eggshells. In 2018 a couple of broiler producers in Sweden that usually never deliver chickens colonised with *Campylobacter* to slaughter, delivered *Camplyobacter* positive chickens. It was in the autumn when we usually have low prevalence of campylobacter in both chickens and humans in Sweden. Whole genome sequencing (WGS) showed that it was the same ST in most of the flocks. Furthermore, some workers at the slaughterhouse got campylobacteriosis with the same ST, ST-148 (folkhalsomyndigheten.se). ST-148 was isolated from four different parent flocks from a farm that deliver eggs to the hatchery that delivered chicken to those producers.

We decided to examine if *Campylobacter* can survive on eggshells and if so, for how long. The results can answer the questions if *Campylobacter* can be transmitted from parent birds to newly hatched chicks if *Campylobacter* are present on the outside of the eggs. Isolates of three different sequence types of *Campylobacter jejuni* were used in the study; ST-257, ST-148 and ST-918. Sequence type ST-257 had

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previously been isolated from the biofilms inside water pipes, ST-918 from a transport crate after cleaning, whereas we isolated ST-148 from parent flocks (Frosth et al. 2020). Eggs were artificially contaminated by dipping in broths of three different concentrations and sequence types of *C. jejuni*, with or without addition of caecal contents. The eggs were stored in a refrigerator and analyzed for presence of *Campylobacter* after 1–14 days. Qualitative analyses were performed according to ISO 10272 with enrichment in Bolton broth and culture on mCCDagar. Some of the samples were also analysed by qPCR. After one day in the refrigerator *C. jejuni* were detected by qPCR from 21 (70%) out of 30 eggs whereas *C. jejuni* were isolated from 16 (18%) out of 90 eggs after enrichment and culture on mCCDA. After ten days storage in the refrigerator *C. jejuni* ST-918 were detected from one egg after enrichment and culture on mCCDA (Table 1). Based on the results of this study, it cannot be excluded that there is a risk of transmission from eggs to newly hatched chicks via eggshells if storage is short and optimal condition for *Campylobacter* are achieved (Rocchio 2020).

Table 1. *Campylobacter jejuni* in eggshells dipped in different concentrations and sequence types of *Campylobacter jejuni*- broth with and without caecal contents and analysed after 1 to 14 days storages in a refrigerator.

Day	Low	Medium	High	Control
qPCR Day 1	**1/10**	**10/10**	**10/10**	0/3
Day 1	0/30	**1/30**	**16/30**	0/9
Day 4	0/20	0/20	0/20	0/6
Day 7	0/30	0/30	0/30	0/9
Day 10	**1/20**	0/20	0/20	0/6
Day 14	0/10	0/10	0/10	0/3

Whole genome sequensing have mostly been used in order to identify possible transmission routes and formulate effective measures to prevent chickens being colonized with *Campylobacter*. The isolates from the environment have been compared with those from the chickens and measures have been implemented and tested during the work, which contributed to the nationwide decrease in *Campylobacter*-positive flocks. A farm investigation with sampling from the environment were performed in the summer 2021 due to the producer had delivered an increased number of chicken flocks with *Campylobacter*. Samples were taken from both the chickens and the environment and *Campylobacter* was isolated from the chickens and the incoming drinking water. Since water is a potential risk factor for chickens and the same sequence type (ST-583) was detected in the drinking water and chicken, UV filter was recommended and installed on incoming water. Furthermore, cleaning of water pipes by flushing by increasing and decreasing the pressure of the water in the pipes ere performed, to facilitate the removal of the biofilm in the pipes. These measures had expected effect and *Campylobacter* had not yet (2022-06-30) been detected in any of the chickens from the producer after these measures.

Discussion

The results obtained in this project indicate that the greatest challenge in preventing colonization of broiler chickens by *Campylobacter* is that there are different reservoirs and transmission routes for chicken colonization within and between different broiler producers. Cattle in a pasture near the broiler houses were a potential reservoir of *Campylobacter* for some producers. Cattle have also been identified as important reservoirs for human campylobacteriosis. Since *Campylobacter* are shed in feces and ubiquitous in the environment, including surface waters, they could be transmitted into broiler houses via vectors such as insects, contaminated water, or via vehicles as aerosols or dust. Insufficiently cleaned transport crates is another risk factor for chickens becoming colonized with *Campylobacter* especially during thinning, when birds in a flock are sent to slaughter in separate batches on two or more slaughter occasions.

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Many animals carry *Campylobacter* in their intestines without showing any signs of disease. The intestines of poultry can be colonized with *Campylobacter* up to log 8 CFU/g faeces. Eggshells may be contaminated with *Campylobacter* after being exposed to faeces during laying, which in theory can be transmitted to humans or newly hatched chickens. There is a lack of knowledge of the risk for humans being infected with *Campylobacter* via contaminated eggshells, and if/how often *Campylobacter* can be transmitted from parent birds to newly hatched chickens via contaminated eggshells. Based on the results of this project, it cannot be excluded that there is a risk for the consumer of becoming infected with *Campylobacter* via eggs, though the risk is small as there are several criteria that need to be met for this to be possible. However, the risk is bigger for staff at packing plants and on farms who handle eggs shortly after they have been laid. There is also a potential risk of transmission from eggs to newly hatched chicks via eggshells if storage is short and optimal condition for *Campylobacter* are achieved.

Cleaning and disinfection (C&D) is important to prevent the transmission of bacteria. The significant differences in bacterial load between the farms after C&D confirm that compliance and effectiveness of C&D protocols vary. Furthermore, the spread of bacterial load after C&D between different occasions on the same farm may differ as well which suggest difficulties in achieving stable result over time. The fact that we could not see any differences in bacterial load between different sampling points in the broiler house can be interpreted as that all the surfaces are equally dirty from the very beginning or that they become equally contaminated after the cleaning stage.

Conclusions

The conclusion of this study is that there are different sources and transmission routes for the colonization of chickens with *Campylobacter* at different broiler producers, and thus individual measures have to be taken at each producer to prevent *Campylobacter* colonization. There are also differences between *Campylobacter* sequence types in their ability to survive in the broiler houses, water pipes, slaughter houses, freezing and in the environment in the kitchen. Freezing reduced the concentrations of *C. jejuni* in broiler meat and meat juice samples, but do not eliminate the presence of all bacteria.

Relevance and recommendations

A novel feature compared with previous studies is that measures were implemented and tested during the project. These contributed to a nationwide decrease in *Campylobacter*-positive flocks from 15.4% in 2016 to 5.3% in 2021. To conclude, there are different sources and routes of *Campylobacter* transmission to chickens from different broiler producers, and individual measures must be taken by each producer to prevent *Campylobacter* colonization of chickens.

At cleaning and disinfection the disinfectant must be chosen based on the specific environment as well as the bacteria it aims to eliminate, since the effectiveness differs between different types of environment, especially agents present in biofilms. It was also shown that the effect of mechanical cleaning couldn’t be replaced by disinfectants alone. Regarding cleaning of transport crates, chemical disinfectant in combination with drying with hot air (dehumidifier) was the most effective treatment. A disadvantage is that this treatment is energy-consuming and would require substantial technical changes to the current cleaning process, increasing operating costs at the slaughter plant. However, considering the contribution of improved cleaning of crates to overall hygiene control within the poultry supply chain and the beneficial effect on animal welfare, the costs may be justified.

The difference in the ability of sequence types of *C. jejuni* to withstand stress indicate a possible difference in their ability to cause disease. The mechanisms against various stress factors, e.g. dehydration, oxygen and freezing, and the ability to cause infection in human epithelial cells should be further studied. The findings will contribute scientific knowledge on why certain strains are more likely to cause outbreaks and form recommendations for prevention of *Campylobacter* in the broiler chicken production.

Previous studies have not found any evidence for vertical transmission of *Campylobacter,* i.e. spread from the hen to the chickens. Despite this, we have found the same sequence types of *Campylobacter* in parent flocks and broiler flocks from different producers. Parent birds are a risk factor that has not been

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noticed before, but since we at several occasions found matching of sequence types of *C. jejuni* between parent flocks and broiler flocks, this has to be further studied to ensure if the transmission of *Campylobacter* from parent birds to chickens exist or not. If a transmission takes place, the transmission routes have to be understood so measures can be taken to ensure that the route of transmission could be prevented.

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Result dissemination:

Scientific publications, *published*	Frosth, S., Karlsson-Lindsjö, O., Niazi, A., Fernström, L. L., & Hansson, I. (2020). Identification of Transmission Routes of *Campylobacter* and On-Farm Measures to Reduce *Campylobacter* in Chicken. *Pathogens (Basel, Switzerland)*, *9*(5), 363. https://doi.org/10.3390/pathogens9050363
	Dzieciolowski, T., Boqvist, S., Rydén, J., & Hansson, I. (2022). Cleaning and disinfection of transport crates for poultry - comparison of four treatments at slaughter plant. Poultry science, 101(1), 101521. https://doi.org/10.1016/j.psj.2021.101521
Scientific publications, *submitted*	Eriksson, D, Råhlén, E., Bergenkvist, E., Skarin, M., Fernström, L.-L., Rydén, J., Hansson, Survival of *Campylobacter jejuni* in frozen chicken meat and risks associated with handling contaminated chicken in the kitchen
Scientific publications, *manuscript*	T. Dzieciolowski, T., Boqvist, S., Rydén, J. & Hansson, I. Evaluation of cleaning and disinfection procedures on poultry Farms
	Moazzami, M., Bergenkvist, E., Boqvist, S., Fernström, L.-L., Frosth, S., Vågsholm, I., & Hansson, I. The effectiveness of cleaning and disinfection in the slaughterhouse. Enumeration of *Enterobacterales* and aerobic count. Comparison of sampling with sponges/swabs, ATP-swabs and dipslides.
Conference publications/ presentations	Frosth S., Karlsson-Lindsjö O., Fernström L-L and Hansson I.**,** Identifying possible transmission routes of *Campylobacter* on chicken farms by NGS MedVet. Pathogens 8-11 October 2018, Prato, Italy

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	Frosth S, Karlsson-Lindsjö O., Fernström L-L, Hansson I. Identifying possible transmission routes of *Campylobacter* on chicken farms by high-throughput sequencing CHRO (20th International Workshop on *Campylobacter*, Helicobacter and Related Organisms), Belfast, Northern Ireland, 8th - 11th September 2019
	Rocchio J., Jansson DS., Frosth S. Fernström L.-L- & Hansson I. Survival of Campylobacter jejuni on eggshells. CampyUK goes global, virtual conference for Campylobacter. Sept 8 - 10 2021.
	Viklund M.T, Moazzami M., Hansson I, Production of *Campylobacter jejuni* in biofilms and sensitivity of various disinfectant substances. CHRO (21st International Workshop on *Campylobacter, Helicobacter* and Related Organisms), Zangzhou China, 14th - 19th November 2022
Other publications, *media etc.*	*Campylobacter* – risk för människor men inte för djur | 10 nov 2020, Medarbetarwebben (slu.se), https://internt.slu.se/riktat/interna-fakultetssidor/vh-fakulteten/karnverksamheterna/forskarutbildning/docentforelasningarna-maj-2020/hansson/
	Kycklinguppfödares åtgärder minskar *Campylobacter.* SLUs kunskapsbank. 18 maj 2021 www.slu.se/forskning/kunskapsbank/publicerat/fjaderfa/kycklinguppfodares-atgarder-begransar-campylobacter/
	Är metoderna för rengöring och desinfektion på svenska slakterier och stycknings-anläggningar tillräckliga för att garantera säkra livsmedel? 4 mars 2021. www.slu.se/fakulteter/vh/forskning/forskningsprojekt/djurslagsoberoende/rengoring-och-desinfektion-pa-svenska-slakterier/
	Kan *Campylobacter* överföras via hönsägg? Tidningen Fjäderfä nr 5 2021
Oral communication, *to sector, students etc.*	Webinarium, doktorandseminarier för Svensk Fågel 26-28 oktober 2021, Tomasz Diezielowski, Konsten att leverera kycklingkött utan *Campylobacter*
	Webinarium, doktorandseminarier för Svensk Fågel 26-28 oktober 2021, Madeleine Moazzami, Rengöring och desinfektion på slakterier
	Reduce the number of *Campylobacter* and other pathogenic bacteria in the ready-to-eat chicken. Ingrid Hansson 28 Oct 2022 virtual, Annual Livs Id meeting
	Svensk Fågels Sommarmöte i Ystad. 2022-06-08, Ingrid Hansson. Åtgärder för att minska andelen kycklingar med *Campylobacter* i Sverige
Student theses	Rocchio, Jeremy, 2021. Kan *Campylobacter* överföras via hönsägg? *C*an *Campylobacter* be transmitted via hens' eggs? Supervisor Ingrid Hansson, Omfattning 30 hp Veterinary Medicine Programme, http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-s-16788
	Viklund, Moa Therése 2021, Produktion av biofilm med MRSA, MRSP eller *Campylobacter jejuni,* samt hur olika desinfektionsmedel påverkar den - Supervisor Ingrid Hansson, Omfattning 30 hp Veterinary Medicine Programme, Epsilon Archive for Student Projects (slu.se) http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-s-17632
	Råhlén, Ella, *2021.* Survival of *Campylobacter jejuni* in frozen chicken breast fillet. Omfattning 30 hp *NY010 Agronomprogrammet* - http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-s-17200
	Eriksson, Daniel, 2021. The risk of handling poultry meat with *Campylobacter jejuni* from the consumer's perspective. Omfattning 30 hp NY010 Agronomprogrammet – livsmedel, http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-s-17199
Other	Referensgruppmöte on-line 2020-11-26

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