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Reducing campylobacter cross-contamination during poultry processing

Final Technical Report

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1 Layman's summary

Approximately 70% of raw poultry-meat in the UK is contaminated by Campylobacter bacteria, which cause high numbers of gastrointestinal infections in the human population. Chickens frequently become colonised with these bacteria during the rearing period, but they do not cause disease in the birds. Campylobacters are transferred to the carcasses during slaughter and processing. Campylobacters are common in the farm environment and in wild birdsand, despite high standards of hygiene, it is very difficult to prevent the birds that are raised in broiler houses being colonised during rearing.Furthermore, free-range and organic chickens almost invariably acquire campylobacters from the environment.

This project studied how Campylobacters are transferred from live birds to carcasses, and what might be done to reduce or prevent this occurrence. A survey of five chicken, two turkey,and one duck slaughterlines indicated that the processing systems were highly automated and similar to each other.There were several points in the lines where contamination of the carcass takes place – particularly the 'scald' tank, the plucking machine and the eviscerating machine. Poultry plants have been designed to process high numbers of birds as rapidly and cheaply as possible. Cleaning and disinfection is generally effective, but cannot be done without stopping production for several hours. Even so, almost as soon as processing resumes, the processing equipment becomes recontaminated.

Completely redesigning the slaughterline would be impractical, so the project team concentrated on finding method(s) that could be used by modifying existing processing lines. Treatments during the process (e.g. by adding disinfectants to the scald water) would not prevent contamination later in the process, so a physical or chemical treatment immediately before the carcasses were chilled was chosen as the most practicable intervention. An extensive literature survey was carried out, and various chemicals that had previously been suggested by the European Food Safety Authority (EFSA) as suitablefor this purpose were identified.These chemicals were tested using a spray rig to treat naturally-contaminated carcasses taken straight off the production line. The substances tested were acidified sodium chlorite (ASC), chlorine dioxide (CD), peroxyacetic acid (PAA) and tri-sodium phosphate (TSP) solutions. Their effect was compared with steam treatment, which had already been investigated by the research team in a previous Food Standards Agency (FSA) project. None of the treatments eliminated Campylobacter entirely, but some reduced numbers on the breast- and neck-skin by about 90%. The most effective agents were ASC and TSP, which both had a similar effect to steam. Rinsing them off after application reduced their effect significantly. Spraying with water alone was not effective, neither was a 'flail washer' present in two of the five chicken-processing plants.

Plants process up to 10 flocks of birds (often each comprising 10 or 20,000 birds) in a day, and it has been suggested that it would be beneficial to process Campylobacter negative (C-) flocks before Campylobacter positive (C+) flocks in order to avoid cross-contamination. Problems with doing this include determining whether a flock is C+ or C- before it arrives at the plant. Several plants were visited where the neck-skins of C- flocks, that were processed after C+ flocks, were examined for numbers of Campylobacters. This was to see how much carry-over (cross contamination) occurred. After the first 100 or so carcasses, the carry-over was very low.

This project concludedthat some reduction in numbers of Campylobacters on poultry-meat could be made by improvements in the processing line. This could be achieved by reducing contacts between carcasses and machinery, or by introducing more water sprays to clean machinery and carcasses before the bacteria attach firmly to the meat or the processing surface..

However, the most effective way to reduce numbers of Campylobacters on poultry meat would be to treat carcasses at the end of the process line. This could be done either with a hot water dip, with steam, or by spraying with a chemical such as ASC.

Information from this project was used to assist in the development of an on-line tool to assist poultry-processing companies and enforcement officers improve the hygiene in poultry plants.

2 Executive Summary

This project was carried out to help the FSA meet its objective of reducing Campylobacter contamination in poultry.

The key scientific objectives of the project were to:

1. Identify a 'typical' poultry processing system and any features present in current lines that are not typical but are likely to influence contamination.
2. Quantify and identify the main contamination paths in current processing.
3. Develop methods for reducing contamination and cross-contamination.
4. Evaluate various intervention steps for reducing contamination and cross-contamination.
5. Identify the key scientific data that could be used to develop a best practice guide.

Data from this project was regularly fed into FSA Project M01045 (Evaluation of data generated by meat plants for current HACCP verification purposes and future slaughterhouse hygiene purposes).

Our results were as follows:

2.1 Objective 1: Identify a 'typical' poultry processing system and any features present in current lines that are not typical but are likely to influence contamination

Six chicken, two turkey and one duck processing lines were surveyed and a report written. In each case the project team followed the production line from lairage to portion cutting, and then interviewed production and management staff. The information gained was used to inform continuing studies of current industrial practice and allow targeting of more detailed experimental evaluative measurements. The results from all chicken plants were further combined into an anonymous description of a 'typical' UK chicken processing plant. There were deemed to be too few turkey and duck plants to form a representative sample. Where applicable the 'typical' chicken plant was contrasted to the turkey and duck lines. The survey also included an assessment of hygiene, disinfection and cleaning regimes, on which a separate report was written. The effectiveness of the most commonly-used commercial disinfectants was tested against a panel of Campylobacter isolates and other bacteria.

The abattoir survey revealed that the techniques used in all chicken processing plants were similar, and that the cleaning and disinfection methods were effective against campylobacters. However, because poultry processing is highly mechanised and is conducted at speeds up to 12,000 carcasses per hour, effective cleaning and disinfection cannot be carried out between flocks, only between shifts – overnight or over the weekend. Also, cross-contamination between adjacent carcasses on the line is unavoidable, and occurs via the machinery and also by direct contact.

2.2 Objective 2: Quantify and identify the main contamination paths in current processing

Extent of cross-contamination from Campylobacrer-positive to Campylobacter-negative (C+ to C-) carcasses. This was first investigated by examining neck skins and caecal contents taken at random from all flocks- processed over a number of days - in two poultry processing plants. The caecal contents were examined to determinewhether the flocks were C+ or C-.

The neck flaps from C- flocks processed after C+ flocks were then examined for numbers of Campylobacters. Numbers of Campylobacters on neck flaps from some C+ flocks were also determined for comparison. Results showed that numbers on the vast majority of neck flaps from C- flocks were <25 cfu g^{-1}, (160/170 were <25 cfu g^{-1}, seven between 25 and 99 cfu g^{-1} and three between 100 and 999 cfu g^{-1}). By comparison, the numbers from neck flaps from C+ flocks were significantly higher (of 105 examined, two (2%) contained <25, ten (9.5%) between 25 and 99, 49 (46.5%) between 100 and 999 and 43 (42%) 1000 or more).

A further investigation was carried out to determine whether the first few carcasses from the C- flocks carried higher numbers of campylobacters than those observed in the previous survey. The experiment was therefore repeated, taking five neck skins from the first ~100 carcasses processed, five from carcasses ~500-600 and five from carcasses ~5000-5100 of all flocks processed over several days and from two different poultry plants. Four C- flocks processed after C+ flocks were identified and the numbers of campylobacters per g neck skin compared with those obtained from carcasses at the same points during processing of C+ flocks. After the first ~100 carcasses, almost all the carcasses from C- flocks had <25 cfu campylobacters g^{-1} neck skin, while numbers on neck flaps of carcasses from C+ flocks remained high throughout 28/56 (50%) exceeding 100 cfu g^{-1}, and 10/56 (18%) exceeding 1000 cfu g^{-1}.

Numbers of *Campylobacter* spp. transferred from Campylobacter positive chickens to their carcasses during processing. Visits were made by a team of eight to chicken processing plants on five occasions. Ten samples of necks or neck skins were taken at each of six points on the line during the processing of four flocks at each visit, and numbers of campylobacters, Enterobacteriaceae capable of multiplying at 41.5°C, and pseudomonads were enumerated. Enterobacteriaceae were included as indicators of Campylobacter contamination, as they were found in similar numbers in the intestine, and not all flocks were colonised with Campylobacters. Pseudomonads were an index of contamination that occurred from the processing environment. They are also important spoilage bacteria and thus affect shelf-life. Most carcass contamination with *Campylobacter* spp. and Enterobacteriaceae was detected after scalding with little obvious increase after plucking, or after evisceration. Contamination with Pseudomonads increased steadily all down the line after scalding.

In order to clarify which process was the most important source of carcass contamination with Campylobacters, batches of chicken carcasses were removed from the line immediately after plucking and dipped in water at 80°C for 20 seconds before replacement on the evisceration line. Control carcasses (processed normally) were taken after evisceration, as well as carcasses that had been decontaminated with hot water after plucking. All were sampled by examination of neck flaps or necks as well as the carcass rinse method. Results showed that plucking and evisceration contributed to a similar degree to numbers of *Campylobacter* spp. and Enterobacteriaceae on the fully processed carcasses.

2.3 Objective 3: Develop methods for reducing contamination and cross contamination

Brain-storming sessions and discussions were held in order to identify the methods most likely to be successful in reducing numbers of Campylobacters on carcasses from Campylobacter-positive (C+) and Campylobacter-negative (C-) flocks.

With C+ flocks the problem is to try to minimise transfer to the finished carcass, of Campylobacters present in high numbers in the intestinal contents and on the feathers of the birds. Practical investigations in processing plants showed that similar proportions of contamination was occurring at two main points - during the scald and pluck stage, and

subsequently during the evisceration steps. It was therefore concluded that in order to significantly reduce the numbers of Campylobacters reaching the carcasses during scalding and plucking would be of little benefit if subsequent processing steps during evisceration contributed a similar numbers. A better system for scalding and plucking and/or cleaning and disinfection all along the line between flocks might be effective in reducing cross-contamination from C+ to C- flocks. However, the studies conducted under Objective 2 showed that there was little cross-contamination from C+ to C- flocks beyond the first few hundred carcasses.

The other clear possibility was to investigate the effect of end-product treatment of the fully-processed carcasses, either immediately before, during or after chilling. The possibilities were to use physical (e.g. steam at atmospheric pressure or dipping in hot water) or chemical (e.g. chlorine, chlorine dioxide, acidified sodium chlorite, ozone, trisodium phosphate, mixtures of peroxy acids).

2.4 Objective 4: Evaluate various intervention steps for reducing contamination and cross-contamination

Cross-contamination from Campylobacter positive to Campylobacter-negative carcasses only occurred on the first few hundred carcasses, and in relatively low numbers. Cleaning and disinfection of the machinery between flocks would reduce cross-contamination to a negligible level, but would not be practicable, and would have no effect on carcasses from Campylobacter-positive flocks.

The plucking and subsequent evisceration process both contribute significantly to the numbers of Campylobacters on carcasses, but decontamination of carcasses immediately post pluck did not result in clearly lower numbers of Campylobacters when examined after evisceration. This indicated that evisceration negated the beneficial effect of post-pluck decontamination. The most effective measure would therefore be to use a physical or chemical treatment immediately before chilling. Heat treatment using steam or hot water had previously been found to reduce numbers of Campylobacters significantly on the outside of poultry carcasses (Corry *et al.*, 2007; James *et al.*, 2007) so several chemicals were evaluated. Investigations were carried out as far as possible on naturally-contaminated carcasses during normal production, taken off the line before chilling and used as soon as possible and treated in laboratory experiments using a specially-constructed rig. The effect of spraying acidified sodium chlorite (ASC), chlorine dioxide (CD), peroxyacetic acid (PAA) and tri-sodium phosphate (TSP) solutions on naturally occurring *Campylobacter*, Enterobacteriaceae and *Pseudomonas* spp. on the breast and neck skin of chicken carcasses was compared.

For analysis, the results were subdivided into six microbe-type/skin-location combinations with each subdivision ranked by: a) cfu remaining after treatment, b) mean reductions, and c) the proportional change in numbers of samples below the limit of detection (LoD).

The three groups of bacteria responded similarly to the chemicals applied. *Campylobacter* spp. were no more susceptible than the other two groups. No single chemical treatment gave the best effect across all subdivisions and ranking methods. Generally, ASC and TSP performed better than PAA with CD and water washing alone having little effect. A 30 seconds chemical treatment was usually more effective than a 15 second treatment. Where only a short (15 second) spray time was possible, ASC was the most effective. If longer treatments were possible, TSP was the most effective choice. Rinsing with water after treatment with chemicals reduced the effectiveness of the chemicals.

2.5 Objective 5: Identify the key scientific data that could be used to develop a best practice guide

The research team collaborated with the team, led by Dr Michael Hutchison, working on FSA Project number MO1045 "Evaluation of data generated by meat plants for current HACCP verification purposes and future slaughterhouse hygiene purposes" (FSA MO1045 Report, 2008). Part of the work carried out in this project identified hazards to product integrity for each stage of processing for each of the four meat species (cattle, sheep, pigs and poultry) processed in the UK. Each identified hazard had a basis that was backed by work undertaken by independent third parties and which had been peer reviewed. This data was used during the development of an on-line best practice guide with a scoring system that rewarded good and best processing practices and could be used by abattoir staff and/or inspection officers to evaluate particular slaughter processes. The system asked a series of questions concerning the processing practices, and scored the answers depending on the perceived influence on the hygienic quality of the meat product. The MO1039 research team assisted with the provision of project outputs prior to publication. In addition, the MO10239 project team evaluated published information concerning poultry and pig processing and donated technical expertise by helping score the relative weights assigned to each step in the slaughter line.

Contents

3 Introduction

Campylobacter spp. are the most common cause of bacterial foodborne enteritis the UK and in most other developed countries (Lawson *et al*., 1999; Friedman *et al*., 2000; HPA, 2005). Raw poultry meat has been implicated as a major source of human infection, due to cross-contamination in the kitchen to other foods eaten without further cooking, undercooking and probably direct hand-to-mouth transfer during food preparation (Cogan *et al*., 1999; Redmond *et al*., 2004). Fifty-six per cent of chilled chicken carcasses at retail in the UK in 2001 were contaminated (FSA, 2003), while similar studies in Wales in 2002 found 71% positive (Meldrum *et al*., 2004) and UK-wide in 2014-2015 found 73% positive, of which 19% contained >1000 cfu *Campylobacter* spp. per gram neck skin (Public Health England, 2015).

Improved biosecurity during the raising of housed broiler poultry is reducing the proportion of Campylobacter infected (C+) flocks reaching the slaughterhouse (Bull *et al*., 2006; Dr A. Thomas, personal communication). However, it is difficult to check the Campylobacter status of flocks on-farm in order to schedule the slaughter of Campylobacter-negative (C-) flocks earlier in the day – before C+ flocks. This is due toflocks becoming infected very late in the growth cycle, as well as to delays in sending and examination of samples. In addition, extensively reared flocks (organic and free range) are almost all infected by the time of slaughter (Heuer *et al*., 2001; Corry, Allen, Jorgensen, unpublished observations). A number of studies have indicated that C-ve flocks processed immediately after C+ve flocks become contaminated with campylobacters (Mead *et al*., 1995; Rivoal *et al*., 1999; Newell *et al*., 2001; Corry *et al*., 2003a; Herman *et al*., 2003; Miwa *et al*., 2003; Hermosilla, 2004). Hermosilla (2004) found flocks processed several flocks after C+ flocks also to be positive (by enrichment of neck skins). Newell *et al*. (2001) and Herman *et al*. (2003) found that strains on carcasses from C- flocks originated not only from preceding C+ flocks, but also from contaminated transport crates. Newell *et al*. (2001) also found that some strains of Campylobacter were more likely to be found on finished carcasses, indicating that they were better able to survive conditions in the processing plant. Stern & Robach (2003) and Allen *et al*. (2007) found varying numbers of Campylobacter on carcasses from C+ flocks, which could not be related to numbers in the caeca, but might be attributed to differences in survival of different strains during processing. Numbers of Campylobacters on C- flocks contaminated during processing or transportation have rarely been determined. Corry *et al*. (2003; Allen *et al*., 2007) found 22/40 carcasses examined from C- flocks processed immediately after C+ flocks to be positive for Campylobacter. However, all but three of these contained less than 2.5 $\log_{10}$ Campylobacters.

Birds from Campylobacter positive flocks arrive at the processing plant with high numbers of Campylobacters, not only in their gut (caeca, colon, with lower numbers in crop, and small intestine), but also on the feathers and skin (Mead *et al*., 1995: Berrang *et al*., 2000). During processing, numbers of all varieties of bacteria, including Campylobacters, diminish overall (Mead *et al*, 1994; 1995; Berrang & Dickens, 2000). However, numbers of Campylobacters fall immediately after scalding, but increase again after defeathering and again after evisceration (Oosterom *et al*., 1983: Izat *et al*., 1988; Mead *et al*., 1995; Allen *et al*., 2007). Contamination during defeathering is mostly due to leakage of gut contents (Berrang *et al*., 2001), and other sources of faecal contamination (e.g. during evisceration) which in turn increases numbers of Campylobacters on the final product (Berrang *et al*., 2004). Mead *et al*. (1995 and MAFF project: CSA 1985) found that numbers of Campylobacters on the final product could be reduced by eliminating unnecessary contact of the carcasses with processing machinery, as well as introduction of extra washing points and chlorine. Addition of chlorine to water in contact with the carcasses would not be an option now, but carcass contact points

can be examined and pin-pointed for removal or special attention between flocks. More effective methods of rinsing or washing could also be investigated, for example; use of better washers for carcasses, use of ultrasonics for continuous shackle decontamination, use of more effective disinfectants during CD (cleaning and disinfection) and use of steam during mid-production breaks. The most difficult machine to clean, which could significantly contaminate carcasses, is the defeathering machine (Mead *et al*., 2000; Allen *et al*., 2003a, b). Effective decontamination of this and the eviscerator between flocks would undoubtedly help reduce cross-contamination from C+ to C- flocks. Investigation of the time of survival of Campylobacters in scald tank water (usually at 52-54°C) would also be of interest, in order to judge the time needed between flocks to avoid cross contamination from this source.

Aerial contamination with Campylobacters occurs in the abattoir, most prevalently near the hanging-on, defeathering and eviscerating areas (Whyte *et al*., 2001; Allen *et al*., 2003a, b). Inhalation of Campylobacters by the birds during hanging-on has been identified as a source of contamination of the air sacs and respiratory system, which spreads to the body cavity during processing (Berrang *et al*., 2003). It is not clear how long aerial contamination persists in any of the three most important areas after a Campylobacter positive flock has passed along the line. Quantitative monitoring of aerial contamination at these three points during a working day, and correlating the results with the Campylobacter status of the flocks passing through, would enable assessment of whether measures to reduce aerial contamination would be beneficial. Cross-contamination during chilling seems to be a minor problem (Allen *et al*., 2000a, b).

Campylobacters are not considered to be particularly resistant to disinfectants or heat (Sorqvist, 1989; Corry & Atabay, 2001), and they are not able to multiply in the environment below about 32°C, or in an aerobic environment. They are unlikely therefore to multiply anywhere in the processing plant, and should be easily killed by heat or disinfectants. They also survive poorly in dry situations and when dried on stainless steel, although they are protected to some extent by organic matter (Cox *et al*., 2001; Kusumaningrum *et al*., 2003). Campylobacters attached to chicken skin are also much more heat resistant than would be expected from *in vitro* studies (Corry *et al*., 2003b; Purnell *et al*., 2004). Campylobacters survive better at low (e.g. in chill) than higher temperatures (e.g. ambient) (Yoon *et al*., 2004). Measures most likely to be effective during CD are therefore likely to involve removal of organic residues, use of disinfectants at relatively high temperatures, and drying of the equipment prior to resumption of processing.

This project analysed in a methodical way the routes by which poultry carcasses from C- flocks become contaminated with Campylobacters (and how Campylobacters are passed from C+ to C- flocks) during processing. This was aided by comparison of results obtained by different poultry processing plants in terms of the numbers of Campylobacters on final product, and the details of processing methods used (**Tasks 1** and **2**). Current knowledge indicates that cross-contamination occurs due to contaminated processing equipment and aerial contamination, and that it might be reduced by more effective CD. This may be particularly effective between flocks, control of airflow, and possibly by methods of decontaminating air. Possible new methods of achieving this would be investigated on rigs; by investigation of equipment available commercially, and by investigating the resistance of persistent strains of Campylobacter to disinfectants and drying (**Task 3)**. **Part 4** of the project used information on variations in processing technology together with new techniques designed to reduce cross-contamination, to assist in modifying commercial lines. The best methods were further tested in commercial plants, and the results assessed to identify the best in terms of reducing cross-contamination and overall contamination with Campylobacter. Finally, a generic HACCP plan

was drafted in consultation with the FSA **(Task 5)**. This project should aid the reduction of contamination in carcasses from C+ as well as C- flocks. Reduction of contamination on carcasses from C+ flocks might be achieved by improved processing techniques (e.g. better functioning of evisceration machines, more in-line water rinses) or by post-process treatments such as hot water or steam, or chemical treatments – e.g. trisodium phosphate, acidified sodium chlorite or chlorine dioxide.

The aim of the project is to reduce the proportion of raw poultry contaminated with *Campylobacter* spp. - as well as the numbers present per carcass - in order to reduce exposure in the UK population . Strict biosecurity during raising of intensively-reared poultry can reduce the proportion of infected flocks, but this strategy has not so far excluded *Campylobacter* spp. from all flocks, and it cannot be used for extensively reared (organic and free-range) flocks. In addition, 'thinning' or partial harvesting of birds increases the risk of introducing infection on the farm. There is therefore a need to devise a strategy for minimising cross-contamination of carcasses from Campylobacter infected (C+) flocks to carcasses from Campylobacter-free (C-) flocks. This could be achieved by various methods; use of separate abattoirs for infected and uninfected flocks, slaughter of infected flocks later in the day than uninfected flocks, disinfection between flocks, modifications to lines in order to reduce transfer of *Campylobacter* spp. via aerosols, and modification of machinery or introduction of novel devices to prevent transfer of contamination between carcasses. Reduction of cross-contamination could also reduce numbers of Campylobacters on carcasses from infected flocks.

The aim of this project was to quantify the degree of cross-contamination (from infected to uninfected flocks) and contamination (from infected flocks to their carcasses), and to devise strategies for minimising or eliminating these. Data from this project was also fed into FSA Project M01045 (Evaluation of data generated by meat plants for current HACCP verification purposes and future slaughterhouse hygiene purposes).

4 Survey of UK poultry processing lines

The overall aim of this project was to develop practical methods to reduce Campylobacter contamination and/or cross-contamination in UK poultry production. Knowledge of the range of current processing lines was a prerequisite for this work to frame the studies and evaluate current 'best practice'. To this end, site surveys of several UK poultry production lines were made. Although the work was mainly concerned with chicken production, turkey and duck plants were also visited to establish any potential technology transfer opportunities. Six chicken, two turkey and one duck processing lines were surveyed. The aim of this survey was to identify common features across a number of plants that are likely to result in contamination of the birds being processed. Its purpose was also to identify existing technology and plant specific features/operations that are designed to reduce cross-contamination. This survey was part of Objective 1/Task 1.1 and was Deliverable D3.

Practices and operations at eight poultry processing plants were recorded including physical parameters, such as temperatures, in order to determine typical and atypical operations in the production of poultry meat. The results indicated that plants are reasonably similar if processing the same species.

This study was carried out to establish a microbiological and physical process baseline that would then enable improvements to both existing and novel practices, which could be developed in order to reduce carcass contamination by campylobacter.

4.1 Survey method

A checklist was prepared covering 50 general points and 27 processes on which operating parameters would be collected. Visits were made, between November 2005 and August 2006, to five chicken, two turkey and one duck plant, representing most major processors in the UK. A diagram of each plant was made and measurements taken for certain process durations and conditions. The assembled information was used to determine:

1. Which plants offer typical processes and operating conditions (for baseline microbiological studies).
2. Which plants offer atypical processes expected to be worth further investigation.
3. How the processes interrelate.
4. What processes, current or novel, might be expected to control or reduce Campylobacter contamination.
5. To give the researchers a sound understanding of how and why these current practices have evolved.

4.2 Survey results

Full survey results are shown in Appendix 1 (Section **Error! Reference source not found.**).

The different species of poultry each have specific processing requirements and direct comparison is not possible. However, there are also similarities. This section generically contrasts the processes seen.

4.2.1 General

4.2.1.1 Typical line speeds

Line speeds in the chicken plants ranged between 6300 – 10500 carcasses/hour across all plants, with the mean line speed being approximately 8075 carcasses/hour. In the two turkey plants line speeds ranged between 2000 – 3000 carcasses/hour, with a mean line speed of 2500 carcasses/hour. In the duck plant the line speed was approximately 2000 carcasses/hour.

4.2.1.2 Typical carcass weights

Carcass weights varied according to species. Chicken carcasses ranged between 1.7 – 2.6 kg across all plants, with mean carcass weights being around 1.9 kg. Turkey carcasses ranged between 2 – 18 kg across both plants visited, with mean carcass weights being around 16.6 kg. In the single duck plant visited carcasses ranged between 2 – 3.8 kg.

4.2.1.3 Typical batches

Most plants irrespective of species batched by lorry-load, with reverse traceability to shed on a timed basis. This may not be precise between lorry-loads. One plant batched birds on farm basis. There is no consistent policy on spaces between batches.

4.2.2 Machines

- Shackle line

All plants had shackle cleaning equipment in place, but the effectiveness was highly variable. Carcass contact with fixed structures, that lines crossed and line crossovers where it was possible for drip to fall from one carcass to another were observed in many plants, particularly those that had undergone modification.

- Ceiling condensation

The majority of plants exhibited condensation that could conceivably fall onto carcasses. The degree of the problem was highly variable, even within the same plant.

- Cleaning methods

The general principles were similar in all plants. Short clean down in breaks with deep cleansing overnight. Precise methods, chemicals, cleaning team organisation and chemical suppliers varied substantially. Refer to individual plant sections for full details.

Many plants dosed chlorine dioxide (ClO_2) into cleaning water.

- General plant repair

Generally, plants were well maintained. Where parts were missing or damaged, product flow was not impeded. In-house modifications to lines/machines/processes were not always to the same level of food grade construction as original equipment.

- Build up of gross debris

Build up of gross debris was apparent on guarded evisceration machinery and to a lesser extent on conveyor belts, particularly in boning and portioning areas of the plants.

4.2.3 Process Line

- Bird arrival

All species used a crate and module system for bird transportation. All plants had automated crate washing systems. Some plants had separate entry and exit routes for lorries.

- Hanging and Stunning

One chicken and one turkey plant used gas stunning, where crates were fed to a nitrogen/carbon dioxide (N_2/CO_2) atmosphere, and comatose birds were hung onto the shackle line. The turkey line eased manual handling by running the shackles close to the stunned carcasses and operatives had only to place legs into shackles and not lift the entire carcass.

The remainder of plants used electric stunning where live birds were hung onto shackles then conveyed to the stunner water bath. The hanging and stunning took place in a subdued lighting zone. Many of these plants used a water spray onto shackles before hanging to lubricate birds' leg into the shackle and to aid electrical contact at stun. All chickens had rubbing contact with a "breast comforter" in order to minimise stress to the birds. Hanging of live turkeys was particularly arduous.

- Throat cutting

All chicken and the duck plants used automated throat cutting equipment using twin rotating cylindrical or plain blades. Both turkey plants used a manual throat cut.

- Bleeding

Bleed tanks for all species were similar. Trough lengths varied between 10 m and 24 m and appeared to be plant, not species, specific.

- Scalding

One chicken plant removed heads before scald tank. Another plant electrically stimulated (ES) before scald.

One turkey plant had automation to scrape debris from base of feet, to pull tail feathers and wash vent before entry to scald tank.

All plants used water bath scalding with mean scalder target temperatures of chicken: 52.5°C, turkey: 55.8°C, and duck: 60.0°C. Scald tank lengths varied between 18 minutes and 40 minutes. Line speeds determined scald duration. Scald tanks were typically refilled each day and acid cleaned weekly. Precise arrangements of tanks varied considerably. Some scald tanks exhibited a substantial defeathering effect.

- Defeathering

All plants used between 3 and 5 rubber finger type defeathering modules in series. Typically, the first module does the majority of feather removal. Some plants had installed screens to try to reduce airborne debris travel.

- Washing

Most plants had a post defeathering wash station.

- Various processes before evisceration (EV)

A variety of operations were carried out in different plants and species before the carcass entered the evisceration phase of processing. For chicken this included: electrical stimulation, foot removal, inspection, and head removal. Turkies required foot removal, whilst for duck this involved waxing and wax removal to complete the defeathering process.

- Rehang to evisceration line

All chicken plants had an automated rehang transfer operation onto the evisceration line. One turkey plant had manual rehang at this stage.

- Evisceration

All species underwent a similar process to remove all internal organs, the head, and feet, although precise number and arrangement of equipment vary considerably even within species types. Additionally, turkeys have the "parson's nose" (tail) removed and ducks have beak removed for specific markets.

Chicken evisceration is carried out with carousel type rotary machines, systematically contacting every carcass. Although water sprays are often incorporated to variously lubricate, wash equipment and wash carcasses, the effectiveness is questionable and gross debris accumulates on much of the guarded equipment. Where evisceration equipment is less guarded and in process cleaning is possible, debris does not build up to such an extent.

Turkeys and ducks were eviscerated in a more manual process than chickens, although mechanisation is used for some operations.

- Chilling

All chicken plants used track chillers with impingement of cold air on the vent. Residence times varied between 50 and 80 minutes with air temperatures -2°C to -4°C where stated. One plant included a water spray of the carcasses before entry to the chiller.

Turkeys and ducks are spin-chilled (i.e. using water) with additional air maturation/chilling operations. Maturation/chilling methods varied, one turkey plant placed carcasses in ice filled Dolavs in a cold store, one turkey plant hung carcasses on racks in the air chiller, and the duck plant used spray chilling.

- Grading

Chickens are graded by weight band. Two plants used automated visual grading to augment weight grading operations. It is common to bulk chickens into bins in grade groups.

Turkeys and ducks did not appear to be graded.

- Portioning and boning

All species are portioned in many different manners according to particular customer orders and market forces.

- Packing

All species are packed in many different manners according to particular customer orders and market forces.

4.3 Conclusions

Six chicken, two turkey and one duck processing line were surveyed. In each case the project team followed the production line from lairage to portion cutting, and then interviewed production and management staff. The abattoir survey revealed that the techniques used in all chicken processing plants were similar, and that the cleaning and disinfection methods were effective against Campylobacters. However, because poultry processing is highly mechanised and is conducted at speeds up to 12,000 carcasses per hour, effective cleaning and disinfection

cannot be carried out between flocks, only between shifts – overnight or over the weekend. Also, cross-contamination between adjacent carcasses on the line is unavoidable, and occurs via the machinery and by direct contact. From the combined site visits the following general process observations relating to potential cross-contamination routes were made.

Many sites used bulk bins to store and transport carcasses after chilling and/or grading. This is a potential cross-contamination point between carcasses. Where air chilling was used (chicken) surfaces were drier, possibly reducing bacteria transfer.However, spin chilled turkey and duck will be relatively wet and cross-contamination transfer is more likely. It was concluded that whilst the influence of carcass surface condition and its effect on transfer can be debated, it is certainly prudent to locate any carcass decontamination intervention before bulk binning to lessen the risk still further.

Equipment cleanliness was certainly identified as a factor for investigation. Some plants believed that dosing equipment spray water with chlorine dioxide (ClO_2) reduced this problem. Whilst stainless steel is specified for most food processing equipment various surface roughness grades exist that will affect cleanability. In-plant modifications may not always be as food grade cleanable as original equipment.

The scald tank was considered a prime suspect for cross-contamination, as all carcasses are pulled through increasingly dirty water as the production day progresses. It was considered that additional interventions, as seen in one turkey plant to remove gross debris from the feet before scalder entry, could warrant investigation for chicken. Furthermore, it was postulated that it is probable that dirty and/or contaminated scalder water trapped in the feathers is carried from the scalder to the plucker, exacerbating cross-contamination problems in the plucker. It was concluded that 'steam' scalding as used in some pork plants could be investigated.

The plucker was identified as another prime suspect for cross-contamination – many studies have shown unwanted airborne contamination is generated and the rubber plucker fingers are in contact with every carcass as it passes through the process. Many rubber fingers exhibit small surface cracks caused by their continual flexing and these crevices could easily harbour Campylobacter and other unwanted organisms. Plucker fingers are considered to be a consumable item by many plants, with routine replacement in planned maintenance. It is unclear whether this is a hygiene measure or a quality measure to maintain plucker efficacy. Waste feathers are typically removed from the plucker zone by a water flume, feathers are filtered out and the water is typically recirculated. The microbiological state of this turbulent flow may affect bacterial levels in the plucker area.

Carousel-type evisceration machines, particularly those guarded from the workforce, are prone to accumulate viscera debris. Since every carcass passes through the machines, this could pose a cross-contamination risk. Whilst the carousel-type machines are well suited to high speed processing, additional carcass washing or continuous machine cleaning may be required.

5 Review of scientific literature

In addition to the site surveys (Section 4), as part of Objective 1/Task 1.1 published research and information from the UK and abroad was reviewed. Two specific reviews were conducted. The first specifically addressed the role of poultry processing steps in Campylobacter contamination. The second updated and expanded the review of chemical interventions carried out for a previous FSA Project (M01019: Physical methods readily adapted to existing commercial lines for reducing pathogens, particularly campylobacters, on raw poultry) and reviewed published information on physical and chemical interventions (with particular respect to their effect on Campylobacter and *Salmonella* numbers).

A summary of the findings of these reviews is discussed below. The full reviews can be found in Appendices 2 and 3.

5.1 Role of processing steps

Although there have been many studies published on microbiological numbers and contamination/cross-contamination during poultry processing, only a total of 84 papers were identified as having specific data on Campylobacter numbers during processing. Of these 84 papers, only 47 papers were found to have sufficient relevant information concerning the role of processing conditions on Campylobacter contamination. It is interesting to note that Adkin *et al*. (2006) were able to identify 159 research papers and findings when systematically reviewing sources and factors of on-farm Campylobacter contamination of chickens.

All relevant information extracted from the interesting studies was compiled in the review. All the stages were detailed with all the numbers and comments about Campylobacter contamination. Although the purpose of this review was to concentrate on chicken processing, it was noted that there was very little data available on the role of processing factors on Campylobacter on other poultry species, such as ducks and turkeys. Where data on these species was found it was included.

Table 1 summarises the data identified on the specific impact of different processing steps on Campylobacter contamination on chicken carcasses.

Table 1. Table gathering all the references used for the following review sorted by processing stages, and specifying their effect on the Campylobacter counts and/or prevalence

Processing stage	Number of papers	Number of studies showing an effect on campylobacter counts and/or prevalence			References
		No effect	Positive effect*	Negative effect**	
Bleeding	2	-	-	-	Alter *et al.* (2005), Abu-Ruwaida *et al.* (1994)
Scalding	14	-	8	-	Genigeorgis (1986), Acuff *et al.* (1986), Alter *et al.* (2005), Baker *et al.* (1987), Hinton *et al.* (2004), Oosterom *et al.* (1982), Oosterom *et al.* (1983), Slavik *et al.* (1995), Burh *et al.* (2005), Laisney and Colin (1993), Abu-Ruwaida *et al.* (1994), Berndtson *et al.* (1996), Berrang & Dickens (2000), Berrang *et al.* (2003), Yang *et al.* (2000)
Plucking	16	-	1	10	Abu-Ruwaida *et al.* (1994), Acuff *et al.* (1986), Alter *et al.* (2005), Baker *et al.* (1987), Berrang & Dickens (2000), Berrang *et al.* (2000), Berrang *et al.* (2004), Berrang *et al.* (2003), Berrang *et al.* (2006), Berrang *et al.* (2001), Oosterom *et al.* (1983), Burh *et al.* (2005), Cason *et al.* (2004), Fluckey *et al.* (2003), Ono & Yamamoto (1999), Rosenquist *et al.* (2006)
Evisceration	12	5	2	3	Acuff *et al.* (1986), Alter *et al.* (2005), Allen *et al.* (2007), Baker *et al.* (1987), Ono & Yamamoto (1999), Oosterom *et al.* (1983), Berrang *et al.* (2004), Abu-Ruwaida *et al.* (1994), Hinton *et al.* (2004), Berrang & Dickens (2000), Fluckey *et al.* (2003), Rosenquist *et al.* (2006)
Washing	12	1	6	-	Abu-Ruwaida *et al.* (1994), Acuff *et al.* (1986), Baker *et al.* (1987), Berrang & Dickens (2000), Izat *et al.* (1988), Ono & Yamamoto (1999), Purnell *et al.* (2003), Li *et al.* (2002), Corry *et al.* (2006), Northcutt *et al.* (2005), Sexton *et al.* (2007), Berrang & Dickens (2000)
Chilling	24	2	17	2	Abu-Ruwaida *et al.* (1994), Alter *et al.* (2005), Allen *et al.* (2007), Baker *et al.* (1987), Acuff *et al.* (1986), Berrang and Dickens (2000), Berrang *et al.* (2004), Bashor *et al.* (2004), Cason *et al.* (1983), Logue *et al.* (2003), Oosterom *et al.* (1983), Oosetrom *et al.* (1983), Burh *et al.* (2005), Sanchez *et al.* (2002), Linblad *et al.* (2006), Wempe *et al.* (0983), Li *et al.* (2002), Li *et al.* (1995), James *et al.* (2006), Dickens *et al.* (2000), Berndtson *et al.* (1996), Stern & Robach (2003), Fluckey *et al.* (2003), Rosenquist *et al.* (2006), Son *et al.* (2006)

Positive effect means that campylobacter counts and/or prevalence decrease during the stage

** Negative effect means that campylobacter counts and/or prevalence increase during the stage

It was found, .that a number of very interesting papers had no information about the total number of samples taken, restricting the degree of confidence that can be associated with this data. Also, different studies have sampled at different points, and in a number of cases skipped processing steps, and differences in sampling techniques make comparisons difficult. The followings graphs, Figure 1 to Figure 5, attempt to compare the results of different studies, according to the sampling units expressed.

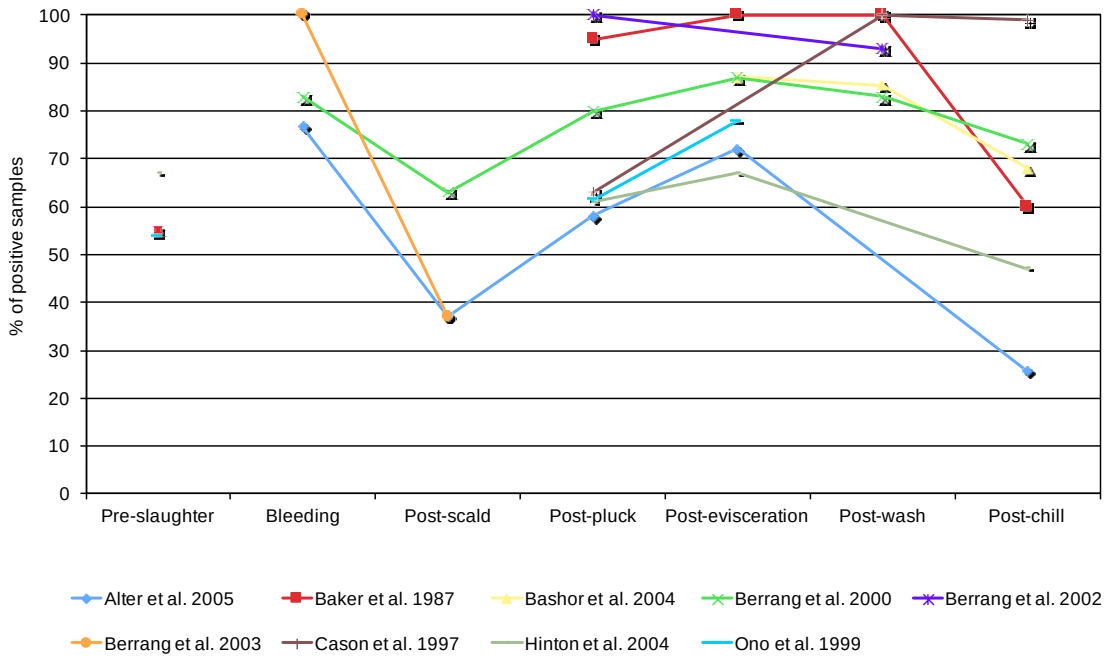

Figure 1. A comparison of published data on the number (%) of Campylobacter-positive samples measured at different processing stages on poultry carcasses reported by different studies

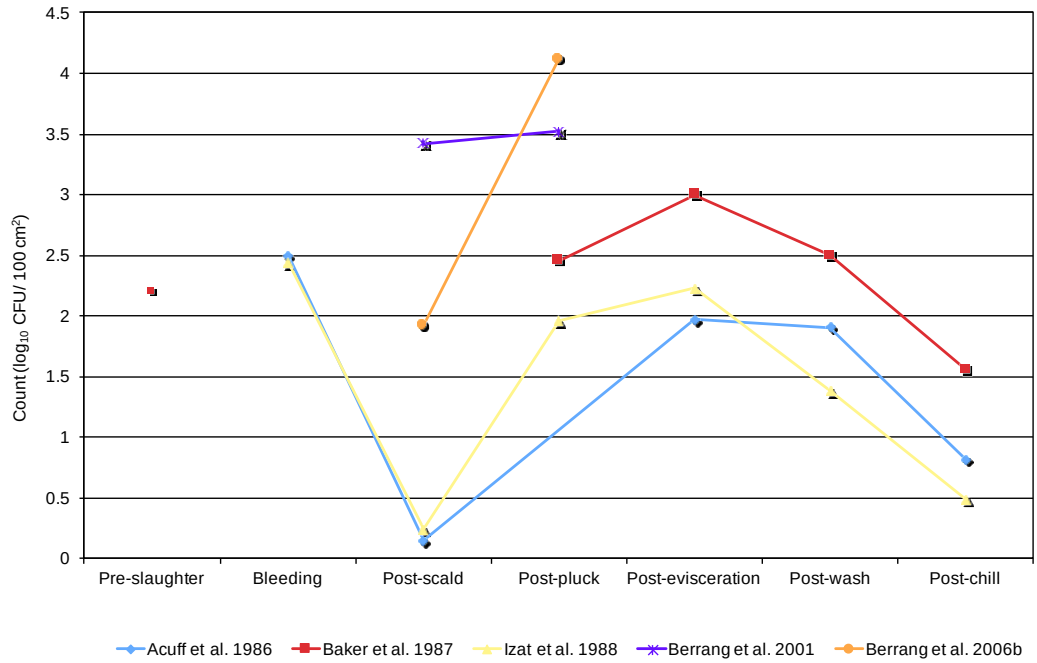

Figure 2. A comparison of published data on Campylobacter counts (log_{10} cfu /100cm^2) measured at different processing stages on poultry carcasses reported by different studies

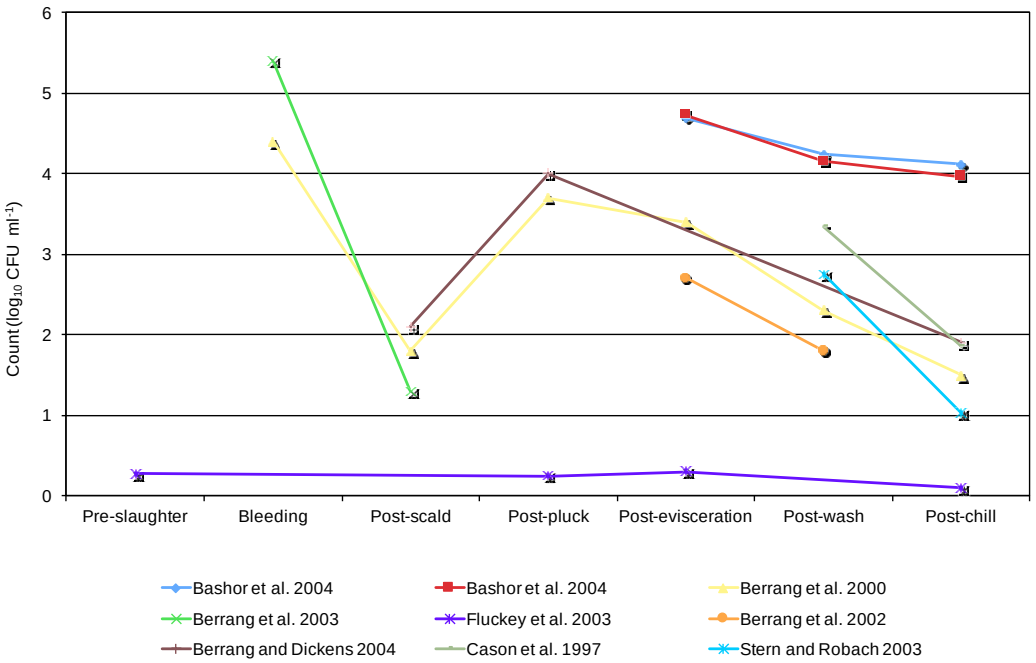

Figure 3. A comparison of published data on Campylobacter counts (log_{10} cfu ml^{-1}) measured at different processing stages on poultry carcasses reported by different studies

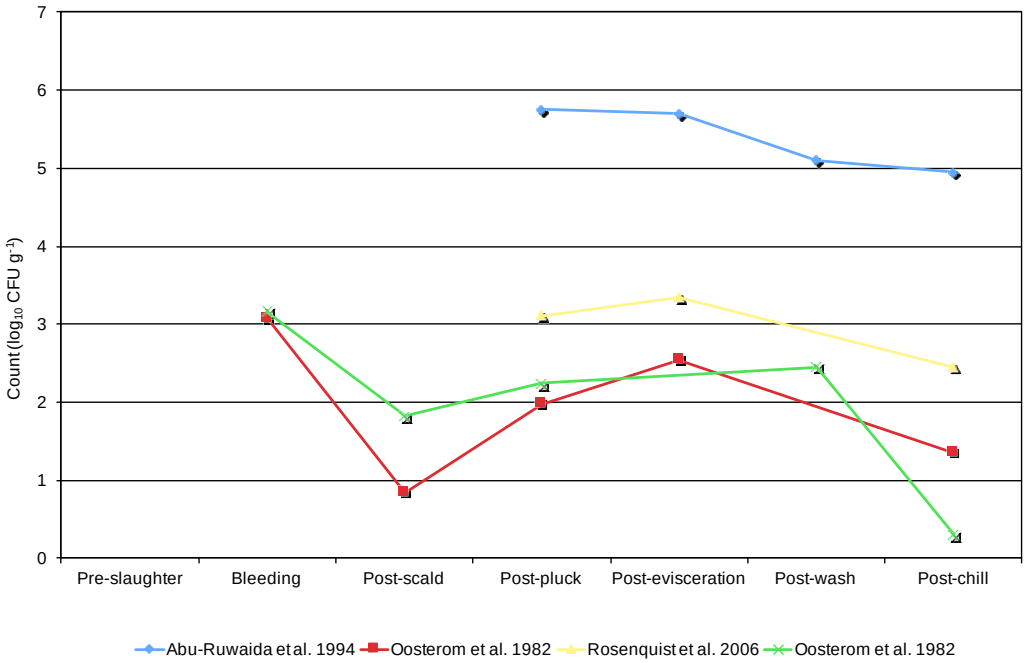

Figure 4. A comparison of published data on Campylobacter counts (log_{10} cfu g^{-1}) measured at different processing stages on poultry carcasses reported by different studies

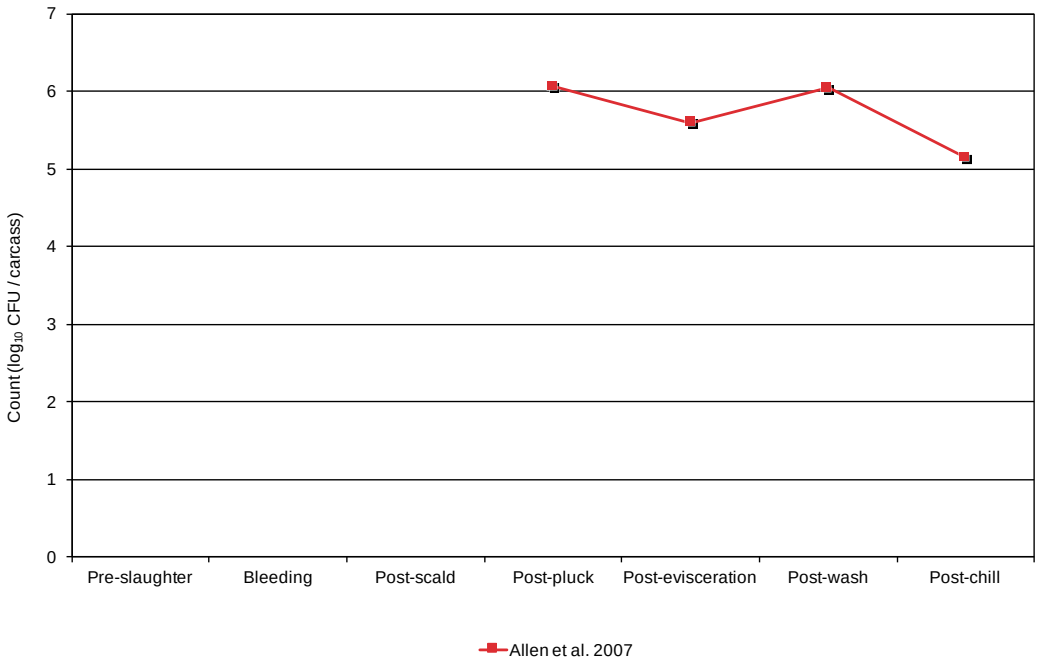

Figure 5. Campylobacter counts (log$_{10}$ cfu /carcass) measured at different processing stages on chicken carcasses (Allen *et al.* 2007).

In general, all these studies show that counts drop after scalding, rise after plucking, continue to slightly rise after evisceration, then drop after washing and chilling.

5.2 Physical interventions

Many publications on a wide range of physical interventions applicable to reducing Campylobacter on poultry carcasses were identified. Some of these interventions have been evaluated on commercial lines (such as washing systems and steam), while others have been applied experimentally to pieces of chicken or chicken-skin (such as microwaves), whilst other interventions (such as visible light) may be applicable but have not been evaluated experimentally. With perhaps the exception of cloacal plugging, there is a greater body of literature on the effects of different physical interventions on red meats than on poultry meat. This literature review generally reviewed only references to the use of an intervention on poultry meat.

5.2.1 Cloacal plugging

A number of studies (Berrang *et al.*, 2001, 2006a and b; Buhr *et al.*, 2003; Musgrove *et al.*, 1997; Russell & Kimball, 2006) have demonstrated that cloacal plugging can reduce microbial contamination during scalding and plucking. Numerous alternative plugging systems have also been patented, including gluing with a cyanoacrylate based adhesive (Neal & Cook, 1996), a tampon-like plug (Lenear & Stockam, 1996), or the use of a highly viscous food grade gel (Anderberg, 2000; Singh, 1997). Some evaluated methods, such as plugging with cotton tampons and suturing, were not considered by the authors to be feasible during industrial operations due to line speeds (Musgrove *et al.*, 1997). There appear to be no independently published evaluations of these different methods, and no specific data on the effect of such interventions on the incidence of Campylobacter on the finished carcass.

5.2.2 Dry heat

Very few studies have investigated the application of dry heat interventions. Cutter *et al.* (1997) had some success on beef. While a limited study on inoculated chicken skin samples, carried out as part of FSA Project M01019 (Physical methods readily adapted to existing commercial lines for reducing pathogens, particularly campylobacters, on raw poultry), showed large reductions in counts of added *C. jejuni* and *E. coli*. Inoculated chicken skin was subjected to 15-minute treatments with high velocity (*ca*. 15 m per second) warm air at 10, 40, 50 and 60°C. Reductions of 1 – 2 log units at 20 and 40°C indicated that there was some form of non-thermal drying effect. However, these data have not been fully validated.

5.2.3 Drying during chilling

There is some evidence that drying the surface of a poultry carcass, which occurs sometimes during air-chilling, may inactivate some of the Campylobacters present (Doyle & Roman, 1982; Oosterom *et al.*, 1983; Bolder & van der Hulst, 1987; Allen *et al.*, 2000a, b). However, this does not seem to be a reliable effect, as confirmed by FSA Project M01019 (Physical methods readily adapted to existing commercial lines for reducing pathogens, particularly campylobacters, on raw poultry).

5.2.4 Freezing, crust-freezing, frozen storage

Freezing and crust-freezing have been suggested as a means of reducing the numbers of Campylobacters on poultry carcasses. Freezing was one of a number of measures taken to reduce the incidence of human Campylobacter infections in Iceland (Stern *et al*., 2003), although its exact contribution is unclear. Freezing to ~-20°C has been reported by a number of studies to result in an initial fall in numbers of Campylobacters, followed by a slower decline during storage (Yogasundram & Shane, 1986; Lee *et al*., 1998; Solow *et al*., 2003; Zhao *et al*., 2003; Bhaduri & Cotterell, 2004; Georgsson *et al*., 2006). Studies appear to show that significant freezing (to temperatures <-20°C) needs to occur in the chicken to see a significant reduction in Campylobacter numbers. Surface freezing has been shown to have only a slight, ≤0.5 log reduction, on Campylobacter numbers (as evaluated in FSA Project M01019 (Physical methods readily adapted to existing commercial lines for reducing pathogens, particularly campylobacters, on raw poultry). Applying this intervention in order to still supply chilled product to the retail market would introduce a considerable bottle-neck to the current production process, since the meat would need to be frozen and then thawed, which would take a considerable number of hours.

5.2.5 Microwaves

A number of studies have studied the use of microwave energy to reduce microbial counts on poultry meat (Teotia & Miller 1975; Cunningham & Albright, 1977; Cunningham, 1978; 1980; Göksoy *et al.*, 1999; 2000) and vacuum-packed beef (Fung & Kastner, 1982; Kenney *et al.*, 1995; Paterson *et al.*, 1995). However, studies at the University of Bristol (Göksoy *et al.*, 1999, 2000) have shown microwave heating to be too uneven and unreproducible to provide an even surface heat treatment capable of producing a significant reduction in Campylobacter numbers without some degree of product cooking.

5.2.6 Infra red

Infra red heating has been used successfully to reduce microbial counts on the surface of pig carcasses after scalding and dehairing operations have been completed (Snijders & Gerats, 1977). Carcasses were exposed for 90 seconds, raising the skin temperature to 150°C. Surface

discolouration and drying occurred, but the appearance later reverted to normal. No references have been found to this method being evaluated for treating poultry carcasses.

5.2.7 Water (cold/hot water)

Spray washing of poultry carcasses with cold water after evisceration to remove faeces, blood, dirt, and other organic material from the surface of carcasses prior to chilling is standard practice in most countries. Table 2 lists all the main publications on washing methods on poultry. Temperature, pressure and time of application have been the main variables studied. In some cases, their efficacy in terms of visual cleaning has also been evaluated. Physically removing bacteria using sprays of cold water alone has been shown to be only partially effective, since attached/entrapped bacteria have been shown to be particularly difficult to remove (Firstenberg-Eden, 1981; Lillard, 1988; 1989; Abu-Ruwaida *et al*., 1994). Interventions that rely on unheated (< 30°C) water produce small changes (usually ≈1 log) in the level of bacterial contamination. The primary reason cited by some for the ineffectiveness of cold water is its inability to lower the water surface tension (Bashor *et al*., 2004; Keener *et al*., 2004). Surfactants have been suggested as a possible solution to this problem. Also, systems that provide a scrubbing action have been recommended. Brush washers, similar to car washes and defeathering machines, that use rubber fingers to clean the outside of carcasses have been described (Keener *et al*., 2004), but no reports of their effectiveness have been located.

Table 2. Publications on the decontamination of raw poultry using hot or cold water (washing)

Method	Reference
Cold water spray	Kotula *et al*., 1967; Mulder & Veerkamp, 1974; Notermans *et al*., 1980; Abu-Ruwaida *et al*., 1994; Bashor *et al*., 2004; Keener *et al*., 2004; Escudero-Gilete *et al*., 2005
Hot water immersion	Dawson *et al*., 1963; Pickett & Miller, 1966; Avens & Miller, 1972; Teotia & Miller, 1972; Cox *et al*., 1974a, b; Morrison & Fleet, 1985; Thomson *et al*., 1979a; de Ledesma *et al*., 1996; Berrang *et al*., 2000
Hot water spray	Thomson *et al*., 1974; Berrang *et al*., 2000; Li *et al*., 2002

When hot water emerges from a spray jet it spreads out and the diverse, separated droplets rapidly lose heat by evaporation. In early trials at Langford (MRI) it was found that the maximum impact temperature on the carcass of a spray placed 30 cm away and supplied with water at 90°C was approximately 63°C. Fan-jets operating at high pressures produced significantly higher impact temperatures than other jet/pressure combinations. It is primarily due to the low surface temperatures that are achieved that limited bacterial reductions are reported with hot water spraying. Surface temperatures of 70°C or over appear to be required to achieve reductions approaching 2 log. Temperatures of this magnitude can be detrimental to surface appearance.

Cold-water immersion interventions invariably involve chemicals. A number of studies have shown that immersion causes swelling of skin and tissues and absorption of more bacteria into deep tissues therefore making them more difficult to remove by washing (Notermans & Kampelmacher, 1974; McMeekin & Thomas, 1978; Thomas & McMeekin, 1982; 1984; Lillard, 1985; 1986; 1989).

Trials in a commercial poultry plant, carried out as part of FSA Project M01019 (Physical methods readily adapted to existing commercial lines for reducing pathogens, particularly campylobacters, on raw poultry), using naturally contaminated carcasses, compared treatments

for 10 seconds in steam with 20 seconds in hot water at 80°C. The appearance of the treated carcasses was assessed visually at intervals until the end of shelf-life, and checks made for Pseudomonads, Enterobacteriaceae and Campylobacters on breast skin. Initial levels of *Campylobacter* spp. were low (~1 $\log_{10}$ cfu cm^{-2}) and variable, but reductions (similar for steam and hot water) of about 2 log cycles were obtained for the other two groups. Numbers of Campylobacters were reduced but not eliminated. Visual assessment indicated that the hot water treatment caused less change in appearance than the steam treatment. Carcasses produced using either treatment could be used for production of 'skin-off' portions. It was considered that changes to appearance of skin-on carcasses or portions would be acceptable to many consumers.

5.2.8 Steam

. If steam is allowed to condense onto the surface of meat, it will rapidly raise the surface temperature. One very attractive feature of condensing steam is its ability to penetrate cavities and condense on any cold surface.

The temperature at which water boils is a function of pressure. At atmospheric pressure, steam will be created initially at 100°C. At pressures below atmospheric (sub-atmospheric), the generation temperature will be lower than 100°C, while, at pressures higher than atmospheric, it will be above 100°C. Generation at temperatures other than 100°C does not substantially reduce the heat capacity of the steam. Treatment temperatures below 100°C are less likely to cause damage to the surface of the carcass, but will require longer treatment times than treatments at 100°C and above. One key disadvantage of both low- and high-pressure treatments is that they are batch systems.

5.2.8.1 Sub-atmospheric steam (<100°C)

A number of studies (Klose & Bayne, 1970; Klose *et al*., 1971; Evans, 1999) have shown that condensing steam at sub-atmospheric pressure is an effective method of reducing bacterial contamination on the surface of poultry carcasses. Steam temperatures of between 75°C and 85°C applied for less than 1 minute can reduce counts by 3-4 log. However, some damage to exposed muscle has been noted. No specific data has been published on the effect of such treatments on Campylobacter on whole chicken carcasses. Applying such a batch treatment on-line at the line speeds utilised in the UK poultry industry would be difficult to engineer, although not impossible.

5.2.8.2 Atmospheric steam (90-100°C)

Atmospheric steam cabinets are utilised in the USA for treating beef carcasses. Although a modified version of this system evaluated in the UK by Whyte *et al*. (2003) was not shown to be particularly effective in treating poultry carcasses without producing some degree of surface damage. Trials in a commercial poultry plant, carried out as part of FSA Project M01019 (Physical methods readily adapted to existing commercial lines for reducing pathogens, particularly campylobacters, on raw poultry), using naturally contaminated carcasses, compared treatments for 10 seconds in steam with 20 seconds in hot water at 80°C. The appearance of the treated carcasses was assessed visually at intervals until the end of shelf-life, and checks made for Pseudomonads, Enterobacteriaceae and Campylobacters on breast skin. Initial levels of *Campylobacter* spp. were low (~1 $\log_{10}$ cfu cm^{-2}) and variable, but reductions (similar for steam and hot water) of about 2 log cycles were obtained for the other two groups. Numbers of Campylobacters were reduced, but not eliminated. Visual assessment indicated that the hot water treatment caused less change in appearance than the steam treatment.

Carcasses produced using either treatment could be used for production of 'skin-off' portions. It was considered that changes to appearance of skin-on carcasses or portions would be acceptable to many consumers.

5.2.8.3 High pressure steam (>100°C)

As with sub-atmospheric steam treatments, applying high pressure steam treatments on-line at the line speeds utilised in the UK poultry industry is difficult to engineer, but not impossible. One such semi-continuous multi-batch system has been developed by Kozempel *et al*. (2000; 2001, 2003a, b) as the Vacuum / Steam / Vacuum (VSV) Process. Field studies of a mobile unit (Kozempel *et al*., 2003a) are reported to have achieved a 1.4-log reduction for *E. coli* and a 1.2-log reduction for Campylobacter on naturally-contaminated carcasses. However, there was extensive mechanical damage caused by the introduction of steam into the cavity (similar problems were encountered in unpublished studies on a similar system at the University of Bristol). In further trials, the mechanical damage was eliminated and a 1.1 – 1.5-log reduction was obtained for *E. coli* K-12 (added artificially), with a total process time of 1.1 s, although this period did not include the time taken to introduce and withdraw the product.

5.2.9 Ultrasound

Sonication has been suggested as a potential way of detaching bacteria from the carcass and increasing the accessibility of chemical treatments. Although 'cavitation' caused by sonication has itself been demonstrated to kill bacteria (Sykes, 1965).

Sams & Feria (1991) found ultrasound (47000 Hz; 200 W output), and ultrasound in combination with lactic acid and/or heat, to be ineffective in reducing surface microbial counts on broiler drumsticks. The lack of antimicrobial effect was attributed to the irregular skin surface providing some degree of physical protection against cavitation. In contrast, studies by Lillard (1993; 1994b) showed ultrasound to be effective in reducing numbers of *Salmonella* attached to broiler skin. No studies on its effect on Campylobacter have been identified.

5.2.10 Ultra-violet light

The potential of ultra-violet (UV) radiation in retarding growth or even killing microorganisms has been known since the latter half of the nineteenth century (Haines & Smith, 1933). Commercial use of UV to extend the storage-life of chilled red meat has been reported from the 1930s onwards (Haines & Smith, 1933; Ewell, 1943).

The lethal effect of UV varies with the intensity of the radiation and the time of exposure. Temperature, pH, relative humidity and degree of microbial contamination influence the lethality of UV radiation (Banwart, 1989). UV has low penetrating power. Areas of shadow, dust in the air and the clumping of microbial cells all have a protective effect.

Purnell & James (2000) found that reductions of up to 1.9 $\log_{10}$ cfu cm^{-2} in APCs could be achieved for skin-on chicken breasts exposed to a 3.4 – 3.7 mW cm^{-2} treatment for 10 seconds.

However, UV radiation alone does not appear to be a particularly suitable intervention method for poultry carcasses, because the low penetration of UV restricts its ability to destroy bacteria located in crevices and follicles. Other surface characteristics of chicken skin may also reduce the effectiveness and uniformity of UV decontamination. However, there is conflicting evidence in relation to the potential of UV for treating chicken portions. To achieve an even exposure at all points on the surface of the meat appears to be the main technical problem. The use of robotics and automation to orientate the meat with the source or move the source(s) over the surface to prevent 'shadowing' needs to be examined.

5.2.11 Other novel physical techniques

A whole range of more novel techniques, such as the use of visible light (Mertens & Knorr 1992), have been suggested for treating poultry meat, and in some cases shown to be theoretically viable alternatives in the future. However, many of these surface treatments suffer from the same 'line-of-sight' problems that occur with ultra-violet radiation treatments.

5.3 Chemical interventions

A wide range of chemicals are known that will kill or severely limit the growth of pathogenic and spoilage bacteria. However, the number of chemicals that are, or may be, approved for food use is severely limited and some may only be effective against specific bacteria. Many studies have been carried out to test groups of chemicals for antimicrobial activity against specific pathogenic and food spoilage microorganisms, for example, Carpenter (1972) and Islam *et al.* (1978). Of the various chemicals tested for effectiveness of inhibiting the growth of *Salmonella in vitro* Carpenter (1972) found that only acetic acid, stannous chloride, citric acid, phosphoric acid, calcium propionate and chlorine produced zones of inhibition (Table 3).

Table 3. Chemicals assessed for effectiveness in inhibiting the growth of *Salmonella Enteritidis* (source: Carpenter, 1972)

Chemical	Concentration	Inhibitoryry
Acetic acid	pH 2.0, 2.5	✓
Butyric acid	pH 2.0, 2.5	
Citric acid	pH 2.0, 2.5	✓
Lactic acid	pH 2.0, 2.5	
Malic acid	pH 2.0, 2.5	
Phosphoric acid	pH 2.0, 2.5	✓
Tannic acid	pH 2.0, 2.5	
Tartaric acid	pH 2.0, 2.5	
Stannous chloride	1%, 5%, 10%	✓
Potassium sorbate	1%, 5%, 10%	
Calcium propionate	1%, 5%, 10%	✓
Chlorine	15 ppm, 20 ppm, 25 ppm	✓

Table 4 lists the publications on the use of various chemicals for treating poultry identified in this review. Of these chemicals, three main groups have been investigated more than any others;organic acids, chlorine and its compounds, and polyphosphates.

Table 4. Publications on the decontamination of poultry using chemicals

Compound	Reference
Acidic calcium sulfate	Dickens *et al*., 2004
Acidified sodium chlorite (ASC)	Mullerat *et al*., 1994, 1995; Ivey, 1999; Kemp *et al*., 2000, 2001; Arritt *et al*., 2002; Schneider *et al*., 2002; Bashor *et al*., 2004; Chantarapanont *et al*., 2004; Oyarzabal *et al*., 2004
Cetylpyridinium chloride (CPC)	Kim & Slavik, 1996; Breen *et al*., 1997; Li *et al*., 1997; Yang *et al*., 1998; Xiong *et al*., 1998; Gueye *et al*., 1999; Arritt *et al*., 2000
Chlorine	Thomson *et al*., 1967; Patterson, 1968; Kotula *et al*., 1967; Dye & Mead, 1972; Mead *et al*., 1975; Park *et al*., 1991; Mullerat *et al*., 1994; Morrison & Fleet, 1995; Bautista *et al*., 1997; Erickson, 1999; Kemp *et al*., 2000; Park *et al*., 2002; Li *et al*., 2002; Fabrizio *et al*. 2002; Bashor *et al*., 2004; Gonçalves *et al*., 2005
Chlorine dioxide	Thomson *et al*., 1967; Patterson, 1968; Kotula *et al*., 1967; Dye & Mead, 1972; Mead *et al*., 1975; Park *et al*., 1991; Mullerat *et al*., 1994; Morrison & Fleet, 1995; Erickson, 1999; Kemp *et al*., 2000; Park *et al*., 2002; Li *et al*., 2002; Fabrizio *et al*. 2002; Bashor *et al*., 2004; Gonçalves *et al*., 2005
Eau Activée	Arpitha, 2005
Electrolysed water	Park *et al*., 2002; Fabrizio *et al*., 2002; Ozer & Demirci, 2005
Ethylenediaminetetraacetic acid	Teotia & Miller, 1975; Mullerat *et al*., 1994; Samuelson *et al.*, 1985
Fatty acids	Hinton & Ingram, 2000; 2003; 2005; 2006; Anang *et al*., 2007
Glutaraldehyde	Thomson *et al*., 1977; Bailey *et al*., 1977; Mast & MacNeil, 1978
Herb extracts	Dickens *et al*., 2000
Hydrogen peroxide	Lillard & Thompson, 1983; Mulder, 1987; Izat *et al*., 1989; Fletcher *et al*., 1993; Wagenaar & Snijders, 2004
Iodophor	Spencer *et al*., 1968
Lysozyme	Teotia & Miller, 1975; Samuelson *et al.*, 1985; Chatzopoulou, 1991
Monochloramine	Russell & Axtell, 2005
Organic acids	Thomson *et al*., 1967; Juven *et al*., 1974; Cox *et al*., 1974b; Thomson *et al*., 1976; Arafa & Chen, 1978; Izat *et al*., 1989; van der Marel *et al*., 1989; Sawaya *et al*., 1995; Tamblyn & Conner, 1997a, b; Ellerbroek *et al*., 1997; Li *et al*., 1997; Zeitoun *et al*., 1997; Bautista *et al*., 1997; Bilgili *et al*., 1998; Xiong *et al*., 1998; Ellerbroek *et al*., 1999; Sakhare *et al*., 1999; Fabrizio *et al*. 2002; Gonçalves *et al*., 2005; Jiménez *et al*., 2005; Anang *et al*., 2006, 2007
Ozone	Yang & Chen, 1979; Sheldon & Brown, 1986; Montecalvo, 1998; Fabrizio *et al*. 2002; Diaz *et al*., 2001, 2002; Enzweiler *et al*., 2002
Peroxyacids	Vadhansin *et al*., 2004; Chantarapanont *et al*., 2004
Phosphates	Elliot *et al*., 1964; Thomson *et al*., 1979a; Lillard, 1994a; Mullerat *et al*., 1994; Slavik *et al*., 1994; Hwang & Beuchat, 1995; Rathgeber & Waldroup, 1995; Hathcox *et al*., 1995; de Ledesma *et al*., 1996; Ellerbroek *et al*., 1997; Bautista *et al*., 1997; Li *et al*., 1997; Colin & Salvat, 1997; Xiong *et al*., 1998; Ellerbroek *et al*., 1999; Kim & Marshall, 1999; Capita *et al*., 2000a, b; Arritt *et al*., 2002; Capita *et al*., 2002; Fabrizio *et al*. 2002; Capita *et al*., 2003; Hinton & Ingram, 2003; Bashor *et al*., 2004; Bourassa *et al*., 2004; Bourassa *et al*., 2005; Gonçalves *et al*., 2005; Del Río *et al*., 2005
PHMB	Islam & Islam, 1979a, b; Thomson *et al*., 1980, 1981
Salt Solutions	Morrison & Fleet, 1985; Li *et al*., 1997; Gonçalves *et al*., 2005
Sodium bisulfate (SBS)	Li *et al*., 1997; Yang *et al*., 1998
Sodium hydroxide	Capita *et al*., 2002
Sorbates	Perry *et al.*, 1964; Robach & Ivey, 1978; Cunningham, 1979; Robach, 1979; To & Robach, 1980; Cunningham, 1981; Morrison & Fleet, 1985; Gonzalez-Fandos & Dominguez, 2007
Sumac	Gulmez *et al*., 2006

The chemicals, or groups of chemicals, discussed in this report that have been investigated in respect to their action on Campylobacters and/or Salmonellas on poultry, are shown in Table 5.

Table 5. Chemicals investigated for their specific action on *Campylobacter* and *Salmonella* spp. on poultry

	Study on the effect of treatment on…	
Compound	*Campylobacter* spp.	*Salmonella* spp.
Acidic calcium sulfate		
Acidified sodium chlorite (ASC)	Yes	Yes
Alkyldimethylbenzylammonium chloride		
Cetylpyridinium chloride (CPC)	Yes	Yes
Chlorhexidine		
Chlorine	Yes	Yes
Chlorine dioxide		Yes
Copper sulfate pentahydrate		
Eau Activée		
Electrolysed water	Yes	Yes
Ethylenediaminetetraacetic acid		
Fatty acids	Yes	Yes
Glutaraldehyde		Yes
Herb extracts	Yes	
Hydrogen peroxide		
Iodophor		
Lysozyme		Yes
Monochloramine		Yes
Organic acids		Yes
Ozone		Yes
Peroxyacids	Yes	Yes
Phosphates	Yes	Yes
PHMB		Yes
Salt (NaCl)		Yes
Sodium bisulfate (SBS)		Yes
Sodium hydroxide		
Sorbates		Yes
Sumac		

Reductions achieved by chemical interventions,specifically on Campylobacters and Salmonellas, are shown in Table 6 and Table 7respectively. The range of "commercial antimicrobials" for processing chickens, with specific regard to *Campylobacter* spp., were reviewed by Oyarzabal (2005).

Overall it can be noted that:

Campylobacter spp. are susceptible to "chlorine based methods" which may be the most effective. Chlorine based methods seem to be more effective against Campylobacter in suspension, provided the organic load is low, rather than by direct application to the carcass surface. Thus these methods would seem to be better for preventing cross-contamination rather than for carcass decontamination.

There is little to choose between chlorine dioxide and acidified sodium chlorite in their effectiveness upon *Campylobacter* spp. as the chemical processes are similar.

Trisodium phosphate efficacy against *Campylobacter* spp. seems to be based on being a strong alkali (high pH). It is more effective at relatively high temperatures and therefore its use in

chilled/chiller sections of carcass processing would be inappropriate. As an alkali it would seem to be inappropriate to use it in conjunction in an acidic environment such as with any of the other three compounds of interest.

Peroxyacetic acid disinfectants are complex mixtures of compounds mixed in prescribed relative concentrations to give commercial products. There may be scope to vary the relative concentrations in order to attack/control specific bacteria.

Greater reductions in bacterial numbers are generally achieved at higher temperatures. In many cases, however, it is difficult to separate the effect of the temperature from any synergistic effect of the chemical. However, there are potential problems with hot chemicals, Reynolds & Carpenter (1974) found for instance that organic acids applied to hot carcasses volatised, creating an irritating atmosphere near the spray.

Some chemical interventions appear to be less effective, either because they achieve only limited reductions in microbial counts or they diminish meat quality. Ozone causes problems arising from its instability and oxidising potential. Hydrogen peroxide has been found to produce unacceptable reactions with catalase from the blood in poultry carcasses. Lysozyme on its own is only effective against Gram-positive bacteria.

The range of operational variables is extensive. These include: temperature, pH, duration of contact, application as a spray or dip and point of application on the processing line.

Table 6. Specific results of chemical interventions in relation to their effect on *Campylobacter* spp. on poultry

Chemical	Treatment	Approximate reduction	Reference
Hot water	Hot water immersion or spray	<0.5 $\log_{10}$ cfu ml^{-1} carcass rinse	Berrang *et al*., 2000
Hot water	Hot water inside-outside washer	0.8 $\log_{10}$ cfu per carcass	Li *et al*., 2002
Hot water (50°C)	Spray (lab based)	-0.3 $\log_{10}$ cfu/skin	Arritt, 2002
ASC	Spray (lab based)	1.5 $\log_{10}$ cfu/skin	Arritt, 2000
ASC	Spray washing	>1 $\log_{10}$ cfu ml^{-1}	Kemp *et al*., 2001
ASC	Spray (lab based)	1.5 $\log_{10}$ cfu /skin	Arritt *et al*., 2002
ASC	Washing	2 $\log_{10}$ cfu cm^{-2}	Alcide, 2002
ASC	Immersion (lab based)	1 log	Chantarapanont *et al*., 2004
ASC	Washing	1.2 $\log_{10}$ cfu ml^{-1} carcass rinse	Bashor *et al*., 2004
CPC	Spray (lab based)	2.9 $\log_{10}$ cfu/skin	Arritt, 2000
CPC	Spray (lab based)	1.4-2.9 $\log_{10}$ cfu/skin	Arritt, 2002
Chlorine	Hot water inside-outside washer	0.8 $\log_{10}$ cfu per carcass	Li *et al*., 2002
Chlorine (electrolysed water)	Immersion (lab based)	3 $\log_{10}$ cfu g^{-1}	Park *et al*., 2002
Chlorine	Washing	<0.5 $\log_{10}$ cfu ml^{-1} carcass rinse	Bashor *et al*., 2004
Fatty acid (Potassium oleate)	Washing	2 $\log_{10}$ cfu ml^{-1}	Hinton & Ingram, 2000
Fatty acids	Immersion (lab based)	>1 $\log_{10}$ cfu ml^{-1}	Hinton & Ingram, 2003, 2005
Protecta II (herb extract on sodium chloride carrier)	Immersion chilling	2.0 $\log_{10}$ cfu ml^{-1} carcass rinse	Dickens *et al*., 2000
Peroxyacetic acid	Immersion (lab based)	1 log	Chantarapanont *et al*., 2004
TSP	Immersion (50°C)	1.5 log	Slavik *et al*., 1994
TSP	Immersion (10°C)	0.2 log	Slavik *et al*., 1994
TSP	Immersion		Salvat *et al*., 1997
TSP	Spray (lab based)	1.6 $\log_{10}$ cfu/skin	Arritt, 2000
TSP	Spray (lab based)	1.6 $\log_{10}$ cfu/skin	Arritt, 2002
TSP	Washing	1.0 $\log_{10}$ cfu ml^{-1} carcass rinse	Bashor *et al*., 2004
Tween 80	Spray (lab based)	0.6 $\log_{10}$ cfu/skin	Arritt, 2002

Table 7. Specific results of chemical interventions in relation to their effect on *Salmonella* spp. on poultry

Chemical	Treatment	Reduction	Reference
Water	Washing	-	Kotula *et al*., 1967
Hot water	Immersion (lab based)		Avens & Miller, 1972
Hot water	Immersion (lab based)		Teotia & Miller, 1972
Hot water	Immersion (lab based)	2 log	Morrison & Fleet, 1985
Hot water	Immersion (lab based)	>2 log	Thomson *et al*., 1979a
Hot water	Immersion (lab based)	0.9 $\log_{10}$ cfu ml^{-1}	De Ledesma *et al*., 1996
ASP	Immersion (lab based)	0.8 $\log_{10}$ cfu ml^{-1}	Mullerat *et al*., 1994
CPC	Spray & immersion (lab based)	1.7 log	Kim & Slavick, 1996
CPC	Immersion (lab based)	4.87 $\log_{10}$ cfu/skin	Breen *et al*., 1997
CPC	Spray chamber (lab based)	1.6 $\log_{10}$ cfu/carcass	Li *et al*., 1997
CPC	Inside-outside washer (lab based)	2 $\log_{10}$ cfu/carcass	Yang *et al*., 1998
CPC	Immersion (lab based)	1.9 log	Xiong *et al*., 1998
CPC	Spray (lab based)	2.9 $\log_{10}$ cfu/skin	Arritt (2000)
Chlorine	Immersion		Thomson *et al*., 1976
Chlorine	Rinsed	75% incidence reduced to 10%	Villarreal *et al*., 1990
Chlorine	Immersion (lab based)	4 log	Park *et al*., 1991
Chlorine (electrolysed water)	Spray	1.1 $\log_{10}$ cfu ml^{-1} of rinse	Fabrizio *et al*., 2002
Chlorine dioxide	Immersion chiller water	14.2% incidence reduced to 1%	Lillard, 1980
Chlorine dioxide	Rinsed	75% incidence reduced to 0%	Villarreal *et al*., 1990
EDTA	Hot water immersion (lab based)	5 $\log_{10}$ cfu ml^{-1}	Teotia & Miller, 1975
EDTA	Immersion (lab based)	0.2 $\log_{10}$ cfu cm^{-2}	Teotia & Miller, 1975
Glutaraldehyde	Immersion	250 cfu/carcass	Thomson *et al*., 1977; Bailey *et al*., 1977
Grapefruit seed extract	Immersion (lab based)	1.8 log	Xiong *et al*., 1998
Lysozyme	Hot water immersion (lab based)	5 $\log_{10}$ cfu ml^{-1}	Teotia & Miller, 1975
Lysozyme	Immersion (lab based)	0.3 $\log_{10}$ cfu cm^{-2}	Teotia & Miller, 1975
Monochloramine	Immersion chilling	8.7% prevalence to 4%	Russell & Axtell, 2005
Organic acid (succinic)	Immersion		Thomson *et al*., 1976
Organic acids (citric, malic, mandelic, propionic, tartaric, lactic and acetic acids)	Immersion (lab based)	2 log	Tamblyn & Conner, 1997a, b
Organic acid (lactic)	Spray chamber (lab based)	1.6 $\log_{10}$ cfu/carcass	Li *et al*., 1997
Organic acid (lactic)	Inside-outside washer (lab based)	2 $\log_{10}$ cfu/carcass	Yang *et al*., 1998
Organic acid (lactic)	Immersion (lab based)	2.2 log	Xiong *et al*., 1998
Organic acid (lactic)	Immersion (lab based)	1.7 $\log_{10}$ cfu g^{-1}	Anang *et al*., 2007
Organic acid (acetic)	Spray	2.3 $\log_{10}$ cfu ml^{-1} of rinse	Fabrizio *et al*., 2002
Ozone	Immersion chiller water	0.7 log	Sheldon & Brown, 1986
Ozone	Spray	1.6 $\log_{10}$ cfu ml^{-1} of rinse	Fabrizio *et al*., 2002
Peroxyacid	Immersion chiller water	-	Vadhansin *et al*., 2004
TSP	Immersion (lab based)	2 log	Lillard, 1994
TSP	Hot water immersion (lab based)		De Ledesma *et al*., 1996
TSP	Immersion (lab based)		Hwang & Beuchat, 1995
TSP	Immersion		Salvat *et al*., 1997
TSP	Spray chamber (lab based)	3.7 $\log_{10}$ cfu/carcass	Li *et al*., 1997
TSP (AvGard)	Immersion		Copen *et al*., 1998
TSP	Inside-outside washer (lab based)	1.78 $\log_{10}$ cfu/carcass	Yang *et al*., 1998
TSP	Immersion (lab based)	2.2 log	Xiong *et al*., 1998
TSP	Spray	2.2 $\log_{10}$ cfu ml^{-1} of rinse	Fabrizio *et al*., 2002
TSP	Immersion (lab based)	2 $\log_{10}$ cfu g^{-1}	Del Río *et al*., 2005

PHMB	Immersion (lab based)		Thomson *et al*., 1980, 1981
SBS	Spray chamber (lab based)	2.4 $\log_{10}$ cfu/carcass	Li *et al*., 1997
SBS	Inside-outside washer (lab based)	2 $\log_{10}$ cfu/carcass	Yang *et al*., 1998
Sorbate	Immersion (lab based)	2 log	Robach & Ivey, 1978
Sorbate	Immersion (lab based)	2 log	To & Robach, 1980
Sorbate	Immersion (lab based)	2 log	Cunningham, 1981

Many chemicals have been applied to meats but not yet investigated with respect to their possible application for reducing Campylobacters and/or Salmonellas on poultry carcasses. There is also an abundance of chemicals where only preliminary laboratory trials have been carried out, and so it is difficult to fully assess their possible effectiveness.

5.3.1 Safety

For any chemical to be approved as a processing aid in the UK and EU it will need to be proved that; (1) no residues are left on treated product that present a health risk, and (2) there is no toxicological effect on the finished product.

Detailed information on the possible toxicological effects of chlorine dioxide, trisodium phosphate, or peroxyacids have been covered by the SCVPH (2003). They note that "*while no residues of the antimicrobial agents have been detected it is known that chlorine dioxide and chlorite do react quickly with organic matter, such as certain amino acids including cysteine, tryptophan, histidine, tyrosine, proline, hydroxyproline and peptides and proteins. Phenols are oxidised to benzoquinones and chlorobenzoquinones… Data on oxidative changes in poultry carcasses due to decontamination with peroxyacids are not available and upon application of chlorine dioxide or ASC for decontamination purposes oxidation of lipids was not detected. The formation of oxidised amino acids or proteins was not investigated. Since possible oxidation products have not been identified a toxicological risk assessment of these products is not possible*." It is clear that while the majority of those chemicals considered for treating meats do not leave residues there are possible concerns regarding possible oxidisation products. In some cases, these concerns have been addressed, however, there are still many chemicals that have been assessed for their possible application in treating meats, but have not been assessed in terms of possible toxicological effects.

In assessing the final exposure risk to the consumer, the SCVPH considered that the daily consumption of 100 grams of poultry should be considered to be a realistic worst-case scenario. In doing so they also noted that "*it must be recognised that preparing poultry for consumption (cooking, frying, etc.) will inevitably reduce the amount of residues significantly, particularly residues of unstable substances like peroxyacetic acid, hydrogen peroxide and chlorite. In other words, poultry ready for consumption will generally contain (much) less residues of unstable decontaminants than the worst case levels*" (SCVPH, 2003). They concluded overall that the risk for adverse health effects for an individual consuming approximately 100 grams of poultry per day decontaminated with chlorine dioxide, trisodium phosphate, or peroxyacids, appeared to be negligible.

The European Food Safety Authority (EFSA) has recently updated the previous opinion expressed by the SCVPH with regard to the toxicological risks of chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids when applied on poultry carcasses (EFSA, 2005a). Their conclusions were:

"*When examining the possibility for reaction products, no halomethanes have been reported to be formed in treatments with chlorine dioxide in water. No chlorinated organics have been found after treatments of poultry carcasses with acidified sodium chlorite. No detectable effects on the oxidation status of fatty acids in poultry carcasses were reported following treatment with peroxyacids. Furthermore, semicarbazide was not detected (limit of detection of 1 microgram/kg) in laboratory tests on poultry carcasses after treatment by immersion with acidified sodium chlorite. The Panel notes that the initial health concerns about semicarbazide are no longer relevant. As set out in previous EFSA opinion, new data showed that semicarbazide is not genotoxic in vivo.*

Based on conservative estimates of poultry consumption in European adults, the Panel estimated potential exposure to residues arising from these treatments.

On the basis of available data and taking into account that processing of poultry carcasses (washing, cooking) would take place before consumption, the Panel considers that treatment with trisodium phosphate, acidified sodium chlorite, chlorine dioxide, or peroxyacid solutions, under the described conditions of use, would be of no safety concern." (EFSA, 2005a).

The EFSA Panel (EFSA, 2005a) favoured spraying as a treatment method since they concluded "*that spraying of poultry carcasses with antimicrobials, by comparison to dipping and immersion treatments, will reduce the exposure to residues and by-products that might arise*".

While chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids have been thoroughly accessed by the SCVPH and EFSA, for many of the other chemicals little publicly published data has been found in the scientific literature that addresses this area. Independent, unbiased and industrially relevant research work is required to aid interpretation and assess the parameters governing these criteria. This will also go some way to addressing many of the meat industry and consumer concerns regarding the use of chemicals.

5.3.2 Costs

Although chemicals are being used in the US

commercially there is very little published data on the cost-effectiveness and cost-benefits of chemical interventions for the treatment of meats, particularly poultry meat.

5.4 Effectiveness of interventions

Many hundreds of publications have been found on the application of physical and chemical interventions to decontaminate poultry and other meats. However, there is rarely any distinction made in the literature between 'decontamination methods', i.e. the whole decontamination system, and 'decontamination treatments'. This often clouds the practical issues of decontamination. There is often too much emphasis placed on the treatment rather than the method of application.

When considering all antimicrobial chemical interventions the method of application must also be considered. In many cases, these are 'drop-in' additions to the washing process rather than an integral part of the washing system. Most chemicals are applied in the form of aqueous solutions. Therefore, as with water interventions the method of application will have a significant influence on how effective a treatment will be. Interventions that rely on unheated (< 30°C) water alone produce small changes (usually ≈1 log) in the level of bacterial contamination. With hot water, surface temperatures of 70°C or over appear to be required to achieve reductions approaching 2 log. Temperatures of this magnitude can be detrimental to surface appearance.

There is also the problem that many of the antimicrobial chemicals described have been investigated in laboratory studies by dipping small samples of meat into solutions of the chemicals. Immersion is a very effective method of ensuring full coverage of a product. However, there are a number of practical problems with immersion, such as maintaining the chemical concentration. As well as being lost through spillage and absorption by the meat, the activity of the solution will change as the chemical reacts with the microorganisms and other organic material. Acid solutions lose activity as the anions are easily bound by peptides and proteins released by the meat (Smulders, 1995). Chlorine also reacts with organic material (Thomson *et al*. 1979b) and ozone (Mackey & Mead, 1990; Sofos & Busta, 1992) and hydrogen peroxide (Sofos & Busta, 1992) in solution rapidly decompose.

Animal carcasses are not ideal shapes to decontaminate. Most decontamination interventions rely on physical contact and uniform coverage of the meat surface. This is difficult, as the surface of many meat products and whole animal carcasses are very irregular. For example, the outer surface of a poultry carcass has many crevices and folds. These areas are commonly considered to be very difficult to treat and provide protection to attached bacteria. They slow down the penetration of aqueous and gas interventions and cause shadowing problems for radiation interventions such as ultra-violet (UV) light. As well as protecting bacteria, these areas often clog up with physical contamination, such as dirt and hair, and do not drain well. Pools of water or chemical solutions lying in these areas can have detrimental effects on the meat appearance and cause difficulties in controlling residence time. However, one recent study contradicts this commonly held belief. Chantarapanont *et al*. (2004) found no evidence of a greater kill at surface sites than in crevices or folds of chicken skin samples inoculated with *C. jejuni* treated with sodium hypochlorite, peracetic acid, and acidified sodium chlorite. They in fact found, "*a greater proportion of viable cells… at the surface than in crevices or folds of treated skin*".

There is much evidence that the time at which products are treated greatly affects the efficacy of decontamination processes. The longer bacteria reside on product surfaces, the more difficult removal becomes, because of the ability of bacteria to attach to tissue. Bacteria differ in their ability to attach to different surfaces and the time they require to become fully attached. The formation of bio-films may increase the resistance of bacteria to disinfectants such as chlorine. Surfactants such as 'Tween 80' have been used to increase surface wetting, in theory allowing the disinfectant to 'get at' the bacteria. 'Tween 80' is not used for food production because it causes unacceptable organoleptic changes. Two surfactants, 'Orenco Peel 40' and 'Tergitol', are used for fruits and vegetables in the USA.

Poultry carcasses typically yield Total Viable Counts (TVCs) between 10^2 and 10^4 cfu per cm^2 (Corry *et al.,* 2003). Obviously, a 4-log reduction would almost guarantee a sterile carcass. To date, no adequate method of achieving this has been found, without affecting the sensory quality of the meat. Also, no treatmentas yet can be relied upon to eliminate all pathogens, although smaller reductions, over and above those achieved in the basic process, would still be of value. In relation to Campylobacter, it has been predicted that a 2-log reduction would lead to a 30-fold decrease in human Campylobacteriosis (Rosenquist *et al*., 2003).

5.5 Conclusions

Of the physical interventions, both hot water and steam have been shown to be effective in reducing Campylobacter on the surface of chicken carcasses. However, there is some concern regarding the effect of either method on the appearance of carcasses and effects on skin elasticity for trussing. Both methods can be readily applied to existing poultry lines. There is

some evidence that plugging the cloaca of carcasses prior to scalding and plucking can have a significant effect on lowering contamination during these processes. However, it is not clear what effect this intervention would have on contamination during evisceration, or how such an intervention could be applied on-line at commercial line speeds.

It is clear from the published studies that the use of chemicals has much to offer in reducing the levels of contamination on the carcass. In addition to the problem of legislation and toxicological issues, there are a number of technical questions that need to be answered before many of these chemicals could be recommended for commercial use. These are:

1. What are the optimum concentrations and compositions of chemical mixtures for different applications or stages in processing?
2. What is the best method of application including time, duration and temperature?
3. What will the cost be and how cost-effective will the treatment be?

This work needs to be carried out under actual, or near to actual, commercial conditions to minimise the problems of extrapolating from laboratory studies. The possible addition of chemicals to washing waters used during evisceration, particularly the inside-outside washer appears the natural place to start.

6 Review of commercial disinfectants

The aim of this review was to review commercially available disinfectants and their relevance with regard to treating Campylobacters and disinfectant-resistant Pseudomonads. This review was part of Objective 1/Task 1.2 and was Deliverable D4/5.

Eight plants were visited between November 2005 and November 2006 – five chicken plants, two turkey plants and one duck plant. Methods of cleaning and disinfection (C&D) of the primary (killing) line between production days and during breaks in production were surveyed, particularly the chemicals used and the supplying companies. Methods of checking the effectiveness of C&D were also surveyed.

Most of the plants surveyed only carried out full C&D between shifts, at other times only removing gross debris and hosing down with water. All plants obtained advice and help in drawing up cleaning schedules from either Holchem (7 plants), Ecolab (2 plants) or Johnson-Diversey (1 plant). Most plants used a similar cleaning system, involving caustic detergent (sometimes chlorinated) followed by rinsing and application of a terminal disinfectant, which was either hypochlorite or QAC (quaternary ammonium compound)-based. The QAC-based disinfectants were Terminol or Holquat (both from Holchem), Triquart-Super (Ecolab), Quatdet Clear (Johnson-Diversey) and Steriklenz 5 (Klenzan). Holquat, Triquart Super and Quatdet Clear all include EDTA as potentiator. Most plants did not rinse off the terminal disinfectant. Most plants checked surfaces after C&D using swabs, a rota of sites and plate counts, with retrospective feedback if results were unsatisfactory.

Swab samples were taken by the research team from the environment in four plants, mostly during production, for *Pseudomonas* and Campylobacter. Isolates from these swabs, and other strains, were tested against three commercially available disinfectants based on quaternary ammonium compounds and one disinfectant based on peracetic acid.

The MICs of four disinfectants (one peracetic acid-based and three QAC-based) were measured, by a doubling dilution method in broth, against a collection of 30 *Pseudomonas* and 15 Campylobacter strains – including those isolated from the poultry plants. The MICs of the Pseudomonads were similar in all four disinfectants, while the Campylobacters were more sensitive (MIC about $1/10^{th}$ that of the pseudomonads) to all the disinfectants except a QAC with nitrilotriacetic acid as potentiator.

According to this test, none of the bacteria examined would have survived if treated with the disinfectants at the recommended concentration. However, the test was carried out on planktonic bacteria, and attached cells would have been more resistant.

The results confirmed the impression that *Campylobacter* spp. are less resistant than *Pseudomonas* spp. to disinfectants, and indicate that cleaning and disinfection properly carried out should inactivate *Campylobacter* spp. in the environment.

6.1 Cleaning and Disinfection and environmental testing in 8 poultry plants

6.1.1 Plant 1 - Chickens (20-Dec-2005 and 18-Aug-2006)

6.1.1.1 Between shifts

There is a team of 12 directly employed by the company. Six work in the primary area (up to the completion of chilling) and six in the cutting area. The slaughter line runs from about 06.00 to 16.00. The cutting line starts at about 4 am. Cleaning starts as soon as production finishes and continues for several hours.

Three main C&D products are used on the machinery on the primary production line, all supplied by Holchem: Holsolve detergent (at 2%), Maxichlor (chlorine-containing detergent – 3%), and Terminol disinfectant (QAT-based disinfectant – 1%). Daily cleaning involves removal of gross debris using low pressure cold water, application of Maxichlor foam, pressure wash (top-downwards) with cold water, hand removal of stubborn dirt followed by water rinse, and final spray with Terminol (left on). Holsolve is used weekly to clean underside and framework surfaces with a scourer.

6.1.1.2 In rest breaks

Gross debris is removed manually and the machinery is hosed down with cold water. No real cleaning or disinfection is attempted. There are no breaks between flocks.

6.1.1.3 Microbiological testing of environment

This is done weekly with a daily rota for a list of 41 sites in the primary production area. Contact (dip) slides are used for TVC and Enterobacteriaceae. "Acceptable" is <10 per cm^2 and unacceptable >10 per cm^2 – with increasing degrees of unacceptability (+, ++, +++). The microbiology testing is done 'in house'. The night cleaning manager takes swabs immediately after cleaning, and more are taken immediately before production starts.

6.1.2 Plant 2 - Chickens (23-Nov-2005)

6.1.2.1 Between shifts

There is a team of 21 staff permanently devoted to cleaning and disinfection overnight. Fifteen work Mon-Fri 22.30 – 06.00 and Saturday 22.30 -09.00, while six work Mon-Thurs 22.30 to 06.00, and Sunday 06.00 to 16.00. This consists of a hot-water (ca. 50°C) hose down. Spray with Holgel Plus (foam containing sodium hydroxide). Soak for 20 min, rinse (the e.v. and first venting machines are done twice with Holgel Plus). The belts are cleaned manually to remove stuck-on dirt, using white scouring pads. Finally, everything is sprayed with 5% sodium hypochlorite – not rinsed.

6.1.2.2 Cleaning during production and in breaks

There are two people employed solely to clean and disinfect in the pluck and evisceration areas. There are six more in the other production areas. These individuals replenish soap, towels, gloves etc, clean floors. During production breaks they hose down with warm water and neutral detergent, then spray with 1% Terminol (hand-held).

6.1.2.3 Microbiological testing of environment

This is done visually as well as using swabs (standard cotton wool swabs in transport medium). Swabs are taken of areas approx. 2.5 x 2.5 cm (1 sq. inch). The swabs are collected daily by Eclipse who examine them for Pseudomonads and TVCs. Counts are reported as <50, >100 up to 10^4 cfu per swab. Results are received about 5 days later. 300 or more colonies are reported to hygiene manager. Sites with 400 or more colonies are reswabbed.

6.1.3 Plant 3 - Chickens (22-Aug-2006)

6.1.3.1 Between shifts

They have their own team of 9 cleaning staff who work 16.30-01.00 Monday –Friday on both slaughter and cutting lines (first slaughter line at 16.00, then cutting line from 21.00). They get their supplies and advice from Holchem. Also, they use Virdine TFR6 (Antec) for disinfecting lorries and transport crates. Routine cleaning schedules use removal of gross debris, hand

removal of more debris, hose down, then application of Maxifoam plus (8% for hard water – caustic – 20 minute contact time) hot water rinse, visual inspection for remaining dirt, with use of scourer and sodium hypochlorite as necessary; spray with 200 ppm sodium hypochlorite and leave for a minimum of 20 minutes before production. The plucker is cleaned only with Maxifoam-plus – no hypochlorite. Checks are only visual on the slaughter line, pre-production swabs are taken in the cutting plant from 49 sites per week, picked randomly.

6.1.3.2 In breaks and between flocks

They only hose down.

6.1.3.3 Microbiological testing of the environment

Microbiological testing is only monitored in the cutting plant. 70 sites are monitored randomly at a rate of 5-6 per day for TVCs using swabs and 10 cm^2 templates. Limit <2000 cfu per swab. Microbiological testing is done by Eclipse.

6.1.4 Plant 4 - Chickens (09-March-2006)

6.1.4.1 Between shifts

Johnson-Diversey (01604 783505, Northampton NN3 8PD) supplies chemicals and advice at the Primary Processing site, which has recently changed its disinfection company from Ecolab. Ecolab currently still serves a secondary meat packing and processing plant.

Overnight cleaning is done (21:30-04:30) by an in-house team (64 persons, including 16 at weekends). Cleaning schedule includes use of a detergent foam (Cleangel –caustic detergent) prior to 1% Quatdet Clear (QAC with EDTA and detergent). The primary production area is divided into 14 areas, with a checksheet for each area. The sections within each area are checked visually for cleanliness (score A (good), B (acceptable) or C (unacceptable) – but in practice A is never used). Machinery is stripped down and deep cleaned using acid in rotation on a monthly cycle at weekends. Weekend cleaning continues in shifts continuously until 04.00 Monday morning, except for all day Sunday, when the engineers make repairs and alterations.

The scalder is emptied and refilled every day, but not cleaned – just flushed with water.

6.1.4.2 In rest breaks

Production staff clean their own lines before breaks. They rinse with water to remove gross debris, then spray with sanitiser spray (1% Quatdet Clear).

6.1.4.3 Microbiological testing of environment

There are 400 designated sites for swabbing environment (post C&D), but only 30 of these sites are covered each month. Numbers of *Pseudomonas* (presumptive) are determined (CFC agar surface plated, 30°C 48 hours), plus TVC (PCA, pour plate 37°C 24 hours), also coliforms, *E. coli* and *Staphylcoccus aureus* (see Table below). The sampling is carried out by QA staff with swabs and diluent supplied by Medical Wire and Equipment Ltd. An area of *ca.* 10 cm^2 is swabbed and the swab placed in 10 ml of diluent (with disinfectant neutraliser). The swabs are left refrigerated for about 2-4 hours before being processed in the lab. Recent results showed that some swabs fail for Pseudomonads (>100 per cm^2) even though the corresponding TVC counts were much lower – whereas one would expect TVCs to be higher than the *Pseudomonas* counts. The reason for this is most likely that Pseudomonads constitute the majority microflora, but do not grow at 37°C. Additionally, Pseudomonads do not grow well in pour-plates. Environmental tests are not done for Campylobacters or Salmonellas.

Test	Method	Pass (cfu per cm^2)	Fail (cfu per cm^2)
TVC	PCA 37°C 24 h	<1000	>1000
Pseudomonas	CFC 30°C 24 h	<100	>100
Coliforms	VRBL 37°C 24 h	<10	>10
E. coli	VRBL 37°C 24 h	<10	>10
Staph. aureus	B-P 37°C 24 h	<20	>20

6.1.5 Plant 5 - Chickens (09-Jun-2006)

6.1.5.1 Between shifts

Production continues 21 hours per day, with only 3 hours for C&D. They use their own staff for cleaning – 3 hours on 5 days per week, and 10 hours on Saturdays and Sundays. They get their supplies from both Ecolab and Holchem.

Holchem:

Chlorfoam - alkaline Detergent

Contact Plus - alkaline Detergent

Holquat - QAC based detergent/disinfectant

Nipac - acid detergent

Ecolab:

Topmax 407- chelated caustic detergent

Herolith- acidic detergent

Topmax 520- acid Detergent

Triquart- QAC-based detergent sanitiser.

Topmax 310- Detergent with active chlorine

Typical cleaning schedules Plucker, no final disinfectant: rinse off gross debris with high pressure water; foam all surfaces with Contact Plus or Chlorfoam; leave at least 20 min; rinse off thoroughly with clean water. (Once per week Contact Plus and Chlorfoam are replaced by Nipac and Holfoam Acid).

Eviscerator with final disinfectant: rinse off gross debris, top downwards and flush through the systems until water is clear; foam all surfaces with Contact Plus or Chlorfoam; leave at least 20 minutes; rinse off thoroughly with clean water; spray all surfaces with Holquat disinfectant (1%). (Once per week treat all surfaces with high pressure hot water and pad all surfaces with a solution of Holfoam acid or Nipac.

Also, they use Virkon-S (Dupont) for disinfecting lorries. Perbac (Ecolab peracetic acid active agent) is used for disinfecting the transport crates.

6.1.5.2 In breaks and between flocks

Only rinsed – 'clean as you go' policy.

6.1.5.3 Microbiological testing of the environment

Seventy sites are monitored at a rate of 5-6 per day for TVCs, Pseudomonads and coliforms, using swabs, and 20 sites are monitored for TVC using contact slides on a rota basis. The

contact slides are incubated and read on site, and the swabs are examined by Alcontrol. The results are plotted to show trends. Limits are as follows:

Environmental swabs- TVC-< 100 per cm^2; *Pseudomonas* - <10 per cm^2 ; Coliforms - <10 per cm^2

Contact Plates - Target - <10 cm^2; Satisfactory - <100 cm^2 ; Unacceptable - >100 cm^2

6.1.6 Plant 6 - Turkeys (08-Jun-2006)

6.1.6.1 Between shifts

C&D is done by a team of 70 employed directly by the company, with 35-40 on the primary production (killing) line. Cleaning is carried out from 20.00 to 04.00, leaving the line to dry for 1.5 hours before the line starts up. Most materials are supplied by Holchem, who provide advice on cleaning schedules. Typical process: remove gross debris; wash with hot water to remove rest of debris; apply Maxifoam Plus (detergent plus sodium hydroxide) or Chlorfoam plus (detergent plus hypochlorite plus sodium hydroxide). Leave for 20 min. Remove remaining marks by hand (abrasive pad) and sodium hypochlorite solution (0.3-0.05%). Rinse with hot water and rinse residue down floor drain. Apply terminal disinfectant (hypochlorite 0.3 – 0.05%); or Holquat 0.5-2%) and leave to air-dry.

6.1.6.2 In breaks and between flocks

C&D not done – cleaning of gross debris and hose down with cold water.

6.1.6.3 Microbiological testing of environment

Ten swabs are taken daily after C&D. These are sponge swabs from TCS or alginate swabs of sticks from Biotrace, both with disinfectant neutraliser. Swabbing is done at random from a list of 120 sites using a matrix. The aim is less than 10^2 TVC per swab (~*ca*. 10 cm^2), $<10^3$ is considered satisfactory and $>10^3$ unsatisfactory. Coliforms are sought in addition to TVC only in the secondary processing line.

6.1.7 Plant 7 - Turkeys (15-Jun-2006)

6.1.7.1 Between shifts

The killing line runs at night and the retail line in the day. The company uses its own cleaning crews 18 evening full time, 14 day full time and 8 in-break cleaners. The retail line is cleaned at night (18 people) and the slaughter line in the day (14 people). Ecolab supplies disinfectants and advises on cleaning schedules. High pressure hoses are used, with low pressure foaming. Two Ecolab QAC-based disinfectants are used: Triquart Super (QAT detergent sanitizer), Tresolin K (detergent sanitizer), and a high foaming chlorinated detergent - Topmaxx 407.

Virkon is used to disinfect transport crates. Typical cleaning schedule involves low pressure hose until all carcasses have been removed; then high pressure water hose to remove all debris; then treat with Topmaxx 407 or Topax 63 foam at 3-5%, leaving in contact for 20-30 min; rinse off thoroughly; spray on Triquart Super at 1-2% and allow to air dry.

6.1.7.2 In breaks and between flocks

They only hose down.

6.1.7.3 Microbiological testing of the environment

The slaughter line has 20 swabbing sites which are swabbed on a 5-sample, 4-week rota (not randomised) on Monday night, post C&D, to be collected on Tuesday A.M by contract

laboratory (Northern Hygiene Laboratories, Driffield) and tested for TVC, coliforms and *Pseudomonas*.

6.1.8 Plant 8 - Ducks (07-Jun-2006))

6.1.8.1 Between shifts

Cleaning and disinfection is carried out daily by contract cleaners. This involves removal of gross debris, hose down with cold water, wash with alkaline detergent and then terminal disinfection with one of the retailer's approved list of disinfectants – Klenzan (QAT-based, www.Klenzan.co.uk) or Terminol (also QAT-based – Holchem). Advice on C&D is provided by Qualitex.

6.1.8.2 In breaks and between flocks

C&D is not carried out in the primary area – only cleaning of gross debris and hosing down with cold water. Sanitiser is used on the cutting line.

6.1.8.3 Microbiological testing of environment

This is carried out using an ATP bioluminescence monitor – ten daily on a rolling basis - and also using swabs (plain cotton wool), with sites rotated on a weekly basis (seven taken each Monday, Wednesday and Friday and sent to a commercial lab). TVC and Pseudomonads are monitored.

6.2 Results of microbiological testing

During some visits swab samples were taken of the environment, sometimes before the start of production (Plant 8), but mostly during production (Plants 4, 5, 6, and 7).

6.2.1 Plant 4 – Chickens (09-Jun-2006)

Five swabs were taken during production. Pseudomonads were isolated from all, and Campylobacters from three.

6.2.2 Plant 5 – Chickens (09-Jun-2006)

Three swabs were taken – all during production: (1) Shackles near evisceration/ shackle before washer; (2) Tray at manual rehang just before chiller; (3) carcass contact point during sorting post-chill.

All were positive for Pseudomonas. Results for Campylobacter were also all positive (by direct plating, but not after enrichment):

6.2.3 Plant 6 – Turkeys (08-Jun-2006)

Four swabs were taken – all during production. All were positive for *Pseudomonas*.

Results for Campylobacter were:

(1) Neck in "dolav"and (2) Greasy deposit on spin chiller spindle: Campylobacter positive by direct plating and negative by enrichment;

(3) Cutting table: (4) Belt for transporting skin in cutting room Campylobacter negative by direct plating and enrichment.

6.2.4 Plant 7 – Turkeys (15-Jun-2006)

Seven swabs were taken, all during production: (1) outside of plucker no.1; (2) floor of empty chiller: (3) drip, post evisceration; (4) breast cone in cutting room; (5) neck-evisceration room;

(6) ACM cutting table *Pseudomonas* positive; (7) belt retail line. All were negative for Campylobacter, and all but nos. 1 and 4 were positive for Pseudomonas.

6.2.5 Plant 8 – Ducks (07-Jun-2006)

Five swabs were taken. Campylobacter was isolated from one (cutting board during production - Campylobacter positive by direct plating and negative by enrichment). Pseudomonas was isolated from 2 out of 3 samples taken pre-production (inside/outside washer, chiller floor) and from both cutting boards sampled during production.

6.3 Summary of abattoir survey results

Most of the plants surveyed only carried out full C&D between shifts, at other times only removing gross debris and hosing down with water. All plants obtained advice and help in drawing up cleaning schedules from either Holchem (7 plants). Ecolab (2 plants) or Johnson-Diversey (1 plant). Most plants used a similar cleaning system, involving caustic detergent (sometimes chlorinated) followed by rinsing and application of a terminal disinfectant which was either hypochlorite or QAC-based. The QAC-based disinfectants were Terminol or Holquat (both from Holchem), Triquart-Super (Ecolab), Quatdet Clear (Johnson-Diversey) and Steriklenz-5 (Klenzan). Holquat, Triquart Super and Quatdet Clear all include EDTA as potentiator. Most plants did not rinse off the terminal disinfectant. Most plants checked surfaces after C&D using swabs, a rota of sites and plate counts, with retrospective feedback if results were unsatisfactory. All plants checked TVCs, 5/8 checked for Pseudomonas, 3/8 checked for Enterobacteriaceae or coliforms, but none checked for Campylobacters.

6.4 Disinfectant resistance of isolates of *Pseudomonas* and Campylobacter

The MIC of four disinfectants manufactured by Ecolab was measured against a collection of 30 *Pseudomonas* and 15 Campylobacter strains – including those isolated from the poultry plants. The MICs were determined using the doubling dilution method in broth according to the recommendations of the British Society for Antimicrobial Chemotherapy (Andrews, 2001). Starting solutions of disinfectants at 1% and 4% were prepared in sterile distilled water on a volume/volume basis and always used within 60 min.

The active ingredients in the first (Topactive®DES) were peracetic acid and hydrogen peroxide, and the other three (Triquart AM, Triquart SUPER and Triquart GB) contained quaternary ammonium compounds (QAC). They are referred to here as A, B, C and D respectively. Disinfectants B and C contained <5% QACs with NTA (nitrilotriacetic acid) and EDTA respectively as potentiators; disinfectant D contained >5% QACs without potentiator. All three QACs had alcohol ethoxylate as preservative at 5-15% in C and D and at <5% in B. While in A, alkyl amine oxide (<5%) acted as a mixture stabilizer. The commercially recommended concentrations for disinfectants A, B, C and D, are 1.0-5.0%, 1.0-1.3%, 1.0-2.0% and 1.0-5.0%, respectively.

The results are listed in Table 8 to Table 10.

Table 8. MICs of four laboratory strains of *Pseudomonas fluorescens* and 20 presumptive *Pseudomonas* strains

Strain	Disinfectant concentration ($\times 10^3$ % (v/v))			
	Topactive®DES	Triquart AM	Triquart SUPER	Triquart GB
CAR1	31	31	63	130
CAR3	31	31	7.8	16
CAR11	31-63	31	7.8	16
CAR12	31	31	7.8	7.8-16
C3	3.9-7.8	31	31	31
C21	31	7.8	31	130
C23	16-31	31	31	63
C22	31	16	7.8	7.8-16
C24	16-31	7.8	31	130
C25	31	16	31-63	130
D1	31	16	63	130
D5	63	31	31	130
D6	31-63	31	16	31
D8	31-63	31	31	63
D9	31	16	31	63
E1	16	16-31	16	130
E5	63	16	16	31
E6	2-3.9	7.8	31	63
F1	63	16-31	16	16
F8	31	31	16	31
F9	16	31	7.8	7.8-16
G1	63	63	31	31
G4	31	63	31	63
G7	7.8	3.9	31	63

F9: identified as *Shewanella putrefaciens* and C3 identified as *Aeromonas* sp.

Table 9. MICs for eight Campylobacter isolates from a laboratory collection (three from poultry) and nine from poultry processing plants

Strain	Disinfectant concentration ($\times 10^3$ % (v/v))			
	Topactive®DES	Triquart AM	Triquart SUPER	Triquart GB
17	3.9	7.8	0.98	0.98
NCTC 11351	3.9-7.8	16-31	0.98	0.98
NCTC 11168	3.9-7.8	31	0.98	0.98
NCTC 13257	2-3.9	7.8-16	0.49-0.98	0.49
NCTC 13255	3.9	31	0.49	0.49-0.98
L6	3.9	7.8-16	0.49-0.98	0.49
EXW30	2	3.9-7.8	0.49	0.49
AR6	0.98-2	16	0.49	0.49
C4	3.9-7.8	63	7.8-16	2-3.9
C71	2	31-63	2	0.98- 2
C72	2	7.8-31	2	0.49-0.98
D1	3.9-7.8	3.9-7.8	0.49-2	2
E1	3.9-7.8	2	0.49	0.49
E2	0.98-3.9	7.8-16	0.98-2	0.24
F1	2	16-31	0.49-0.98	0.49-0.98
F2	7.8	16-31	0.98-2	2
F3	3.9	16-31	2	0.49

Table 10. Comparison of MICs (minimum inhibitory concentration %v/v) of the pseudomonads and campylobacters isolated from poultry processing plants

Bacteria	Top active DES	Triquart AM	Triquart Super	Triquart GB
Pseudomonas	0.0078 – 0.063 (7.8-63)*	0.0078-0.063 (7.8-63)	0.0078-0.063 (7.8-63)	0.0078-0.13 (7.8-130)
Campylobacter	0.00098 - 0.0078 (0.98-7.8)	0.0078-0.063 (7.8-63)	0.00049-0.002 (0.49-2.0)	0.00024-0.0039 (0.24-3.9)

* MIC x 10^3

The MICs of the Pseudomonads were similar in all four disinfectants, while the Campylobacters were more sensitive than the Pseudomonads (MIC about 1/10th that of the Pseudomonads to all the disinfectants except Triquart AM).

According to this test, none of the bacteria examined would have survived if treated with the disinfectants at the recommended concentration. However, the test was carried out on planktonic bacteria, and attached cells would have been more resistant.

The results confirmed the impression that *Campylobacter* spp. are less resistant than *Pseudomonas* spp. to disinfectants, and indicate that cleaning and disinfection properly carried out (especially using the correct concentration of disinfectant) should inactivate *Campylobacter* spp. in the environment.

7 Contamination routes: Contamination from Campylobacter-positive to Campylobacter-negative carcasses – directly and via processing equipment

In spite of the application of biosecurity measures during the rearing of poultry, which has been fairly successful in preventing infection of the birds with *Salmonella* spp., a significant proportion of intensively reared flocks continues to become colonised with *Campylobacter* spp., particularly in the summer. Also, biosecurity measures are difficult to apply to free-range and organic poultry reared with access to the outdoors, with the result that they are almost always colonised with Campylobacters by the time of slaughter (Heuer *et al*., 2001; Avrain *et al*., 2001a, b).

7.1 Materials and methods

7.1.1 Part 1

This study was carried out at chicken processing plant number 4 from March to June 2004. Thirty-two flocks were targeted as potentially Campylobacter-negative (C-). A "flock" was defined as all the birds inhabiting one poultry house or shed (up to about 30,000 birds). Each flock was transported in lorry-loads of 6,000-8,000 birds. Often more than one flock was examined from the same farm.

Caeca. Ten pairs of caeca were taken at random from each flock after post mortem inspection, and divided into two groups of five. Approximately 2 g of the contents from one of each pair of caeca per group of five were mixed and streaked onto mCCDA (modified cefaperazone charcoal deoxycholate agar, Oxoid CM739 plus SR155) plates, and incubated for 48 hours at 42°C in microaerobic atmosphere using sachets (Campygen, Oxoid CN0025A).

Neck flaps. Ten neck flap samples were taken from every target flock or load after chilling. They were taken randomly over as long a time period as possible, and stored at 5±1°C, but only examined for Campylobacters 48 hours later, if the caecal contents were C- and if the last flock from the preceding farm was C+. Presence/absence of Campylobacter was determined by enriching 10 g neck skin in 100ml Bolton broth (LabM L135, with antibiotic supplement, LabM X132, and 5% horse blood, lysed by freezing). The mixture was homogenised using a Stomacher 400 (Seward, London) for 1 minute, and then incubated at 37°C for 48 h in closed containers with small headspace, before streaking onto mCCDA. Additionally, 0.2 ml of the initial suspension was plated directly onto duplicate mCCDA plates for a colony count. All plates were incubated for 48 hours at 42°C in microaerobic atmosphere as above.

Confirmation of suspect colonies. Presence of Campylobacter was confirmed from typical colonial morphology (cream to grey, moist, slightly oily, flat and spreading), oxidase test (+) and morphology by Gram stain of at least one isolated colony per plate (small slender Gram negative curved rods).

7.1.2 Part 2

7.1.2.1 Sampling

A poultry processing plant was visited on four occasions between October 2008 and January 2009. As many flocks as possible were sampled during the production day.

Caeca: Sixteen pairs of caeca per flock were taken, each pair placed in a separate bag.

Neck flaps: Neck flaps were taken from carcasses while still on the line; after the inside-outside washer and before chilling during the processing of each flock. Five (no. 1-5) were taken from - as far as possible - the first 50 carcasses of that flock, five (no. 6-10) at around carcass no. 500 and five (no. 11-15) at around carcass 5000 (total 15 per flock). Each neck flap was numbered consecutively and put into a separate plastic (stomacher 400) bag.

The caeca and neck flaps were chilled immediately and transported chilled to the laboratory and stored at 3 ±1°C until examination.

7.1.2.2 Microbiological examination

The following day, five of the caeca from each flock were examined by preparing a 1 in 10 suspension of caecal content in MRD (maximum recovery diluent, Oxoid) and streaking onto mCCDA. The plates were incubated in microaerobic atmosphere at 41.5°C for 24 hours. If the plates appeared to be negative for Campylobacters, all 16 caecal contents were examined by preparing further decimal dilutions and spreading 200 µl volumes onto duplicate plates of mCCDA, incubating in microaerobic atmosphere at 41.5°C for 48 h.

Neck flaps from flocks with campylobacter-negative (C-) caeca following flocks with campylobacter-positive (C+) caeca were weighed, 50 ml sterile MRD added, and the mixture was homogenised in a Stomacher-400 (Seward, London) for 1 min. Decimal dilutions were prepared in MRD and 200 µl volumes spread onto duplicate plates of mCCDA, incubating in microaerobic atmosphere at 41.5°C for 48 hours.

Colonies suspected to be Campylobacters were checked by oxidase reaction (positive) and for positive reaction using Microscreen Campy latex agglutination (Microgen, Camberley, UK). In some cases, neck flaps from C+ flocks were also examined for comparison.

7.2 Results

7.2.1 Part 1

Table 11 summarises the results. Numbers on neck flaps from C- flocks taken at random during processing were almost always <25 cfu g^{-1} (160/170 were <25 cfu g^{-1}, seven between 25 and 99 cfu g^{-1} and three between 100 and 999 cfu g^{-1}). Whereas those from neck flaps from C+ flocks were significantly higher (of 105 examined, two (2%) contained <25, ten (9.5%) between 25 and 99, 49 (46.5%) between 100 and 999 and 43 (42%) 1000 or more).

Table 11. Number of Campylobacter (cfu g^{-1}) detected on neck skins from C- flocks processed one or more flocks later than a C+ flock (Part 1)

Experiment (load number)[1] and date		*Campylobacter* spp. (cfu g^{-1})			
		< 25	25 – 99	100 – 999	>1000
2	22/3/04	20	-	-	-
3	24/3/04	30	-	-	-
7 (4)	13/5/04	9	1	-	-
7 (5)	13/5/04	6	3	1	-
8	20/5/04	10	-	-	-
9 (7)	21/5/04	5	3	2	-
9 (8)	21/5/04	10	-	-	-
10	25/5/04	30	-	-	-
11	25/5/04	20	-	-	-
15	8/6/04	10	-	-	-
18	9/6/04	20	-	-	-
Totals		170	7	3	0

[1] Load number is specified when colony counts were obtained by direct plating

7.2.2 Part 2

A further investigation was then carried out to determine whether the first few carcasses from the C- flocks carried higher numbers of campylobacters than those observed in the previous survey. Table 12 summarises the results. Four C- flocks processed after C+ flocks were identified and the numbers of Campylobacters per g neck skin compared with those obtained from carcasses at the same points during processing of C+ flocks. After the first ~100 carcasses, almost all the carcasses from C- flocks had <25 cfu Campylobacters g^{-1} neck skin, while numbers on neck flaps of carcasses from C+ flocks remained high throughout 28/56 (50%) exceeding 100 cfu g^{-1}, and 10/56 (18%) exceeding 1000 cfu g^{-1}.

Table 12. Numbers of Campylobacters on neck flaps from Campylobacter negative flocks following Campylobacter positive flocks compared to numbers on neck flaps from Campylobacter positive flocks (log_{10} cfu g^{-1} neck skin or g^{-1} caecal contents) (Part 2)

Campylobacter status of flock		Sample numbers		
		1-5	6-10	11-15
C- after C+ (>9)* 15.12.08	≥1,000	1	0	0
	100-999	1	0	0
	25 - 99	0	1	0
	<25	3	4	5
C+ (8.28)* after C- 15.12.08	≥1000	2	1	
	100-999	2	4	1
	25-99	1	0	
	<25	0	0	
C-, status of previous flock not known 15.12.08	<25	5	5	5
C+ (7.96)* after C+ (6.43)* 12.01.09	≥1000	2	0	0
	100-999	0	1	2
	25-99	3	2	2
	<25	0	2	1
C-, after C+ (7.96)* 12.01.09	≥1,000	0	0	0
	100-999	0	0	0
	25-100	3	0	0
	<25	2	5	5
C+(7.13)*, after C- 12.01.09	≥1,000	0	1	0
	100-999	1	3	4
	25-99	4	1	1
	<25	0	0	0
C- after C+ (7.13)* 12.01.09	≥1,000	0	0	0
	100-999	1	0	0
	25-99	1	2	0
	<25	3	3	5
C+ 30.01.09 first in day (2.85)*	≥1,000	1	2	1
	100-999	4	2	4
	25-99	0	1	0
	<10	0	0	0

* cfu *Campylobacter* spp. per g caecal contents

7.2.2.1 Investigation of the contamination of the environment before the start of and during production

Sampling processing machinery was not possible while the line was moving, but it was possible to take samples of water running off the machinery, and also water from the scald tank. Some swabs were also taken from the processing machinery early in the morning, after overnight cleaning and disinfection, before the line started up. All the water sampled before the start of production was negative (<10 cfu ml^{-1}) for Campylobacters and Enterobacteriaceae. The scald water continued to be negative for Campylobacters during the processing of four C+ flocks, although Enterobacteriaceae were detected. Swabs taken from visibly clean parts of machinery before the start of production normally yielded <10 cfu ml^{-1} MRD. (Stick swabs were placed in 10 ml MRD and agitated on a vortex mixer before surface plating onto mCCDA and VRBG agar.) Swabs taken from visibly dirty areas did yield Enterobacteriaceae and sometimes also Campylobacters.

7.2.2.2 Counts on carcasses at the start of production

Numbers of cfu g^{-1} cCampylobacters, Enterobacteriaceae and aerobic plate count (plate count agar 48 hours 30°C) were determined on 8 samples of 10 neck flaps taken at approximately equal intervals during the processing of the first flock of the day (C+). Each sample was taken while about 200 carcasses passed. The results are summarised in Table 13. No significant difference was found between any of the samples, indicating that numbers had reached a plateau during the processing of the first 200 carcasses.

Table 13. Numbers of *Campylobacter* spp., Enterobacteriaceae and aerobic plate count at 30°C from eight samples of ten neck flaps taken in sequence immediately before chilling from the first flock to be processed on a Monday morning

	Bacterial count (log_{10} cfu g^{-1})		
Sample no.	*Campylobacter* spp.	Enterobacteriaceae	Total aerobic colony count at 30°C
1	3.31	3.95	5.18
2	3.31	4.45	5.46
3	3.26	4.01	4.84
4	3.30	3.98	4.61
5	2.91	4.25	4.88
6	3.09	3.75	4.63
7	3.24	3.98	4.92
8	3.19	3.50	4.42

8 Contamination routes: Numbers of *Campylobacter* spp. transferred from campylobacter positive chickens to their carcasses during processing

The aim of this work was to examine the extent to which different processing stages contribute to contamination when processing Campylobacter-positive flocks. We also investigated to what extent contamination of carcasses during processing is related to the number of campylobacters found in the caeca of the birds at slaughter.

8.1 Materials and methods

8.1.1 Processing plants and flocks

Carcasses from flocks slaughtered in three processing plants (two belonging to the same company) in England were examined during 2006 and 2007. The plants had line speeds of 100-160 carcasses per minute, and were similar in design, with automated lines passing through the stunner, neck cutter, bleed out, scalding tanks (53±1°C), a bank of disc defeathering machines, eviscerater, vent opener, cropper, neck cracker and puller and lung remover. Carcasses were sampled at each point approximately ten minutes after the carcasses started passing, thus all samples were taken from carcasses processing at about the same time. Each flock sampled originated from a single lorry-load – usually one house on a farm.

8.1.2 Detection of Campylobacters

See Section 6.1.

8.1.3 *Detection of Enterobacteriaceae*

Suspensions prepared for enumeration of Campylobacters were plated in parallel onto VRBG agar (Oxoid) and incubated aerobically at 41.5±1°C for 18-24 hours.

8.1.4 Sampling of caecal contents and necks or neck skins

Caeca see section 6.2

Necks (with or without heads) or neck skins were removed from the carcasses as they passed on the production line. This was done using inverted sterile stomacher 400 bags to grasp the neck or neck skin with one hand, while cutting off the neck or neck skin using pruning shears or scissors with the other. Up to 20 samples were taken at each sampling point. For neck samples a piece 6 cm long was cut from each neck and placed in a new stomacher 400 bag. Fifty ml of MRD was added and the mixture pummelled for 1 minute, using a 'Lab-Blender 400' stomacher (Seward, London, UK).

The weight of each neck flap was recorded before adding 30 ml of MRD and the mixture also stomached for 1 min. The liquid phase was used as the initial suspension. The results for the necks were expressed as numbers per ml of the initial suspension. For the neck skins the results were adjusted, using the recorded weights, to be numbers per g neck skin.

8.1.5 Analysis of results

Analysis of differences in the Campylobacter numbers between sets of samples was performed using Student's t-test.

8.2 Results

8.2.1 Investigation of contamination levels in relation to levels in caeca

Comparison for flocks with caecal levels >6.5 $\log_{10}$ demonstrated that levels of campylobacters in neck skin samples correlated with numbers in caeca (Figure 6).

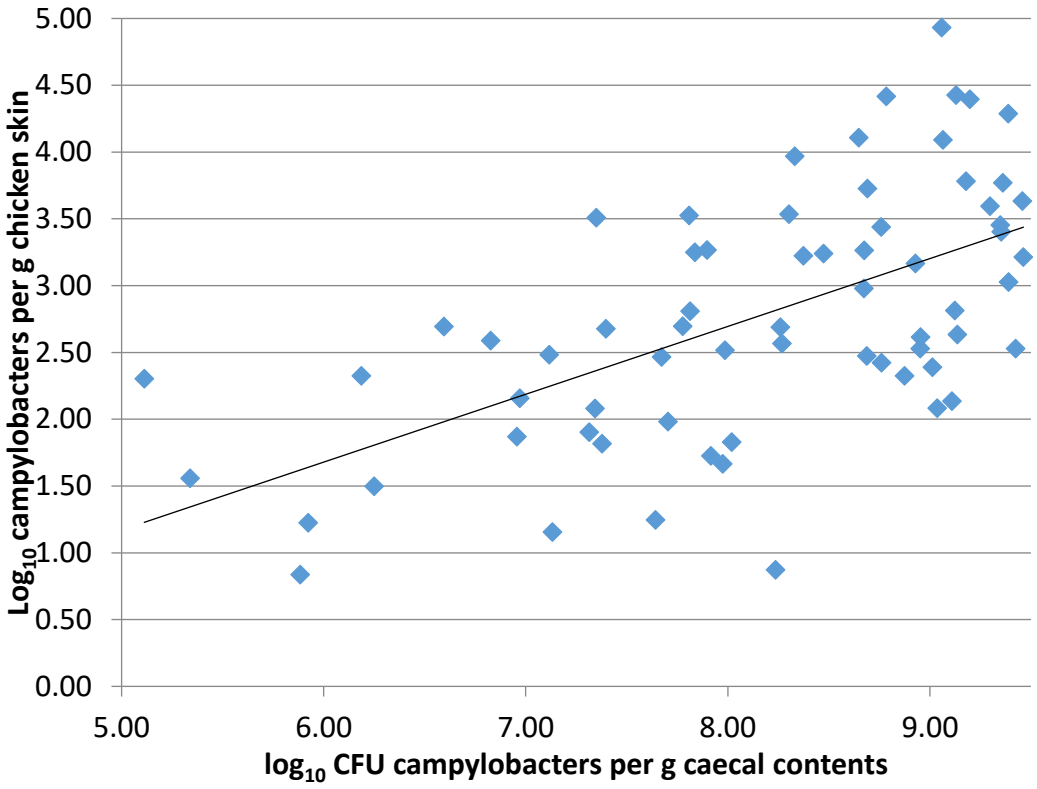

Figure 6. Levels of contamination in neck skin samples after final wash in relation to levels in caecal contents. This includes data from Chapter 8 as well as chapter 7. Each datapoint represents one flock.

However, while in most flocks carcass contamination reflected the level of Campylobacters found in caeca contents this relationship was not proportional for some flocks (Figure 7 to Figure 10). There was good evidence that processing a highly contaminated flock did not result in increased contamination of a subsequent flock. Campylobacters were not detected in neck skin samples from several negative flocks processed immediately after a positive flocks (Figure 9 and Figure 10). There was no obvious effect of the number of birds processed with positive caecal contents on the contamination of a subsequent flock.

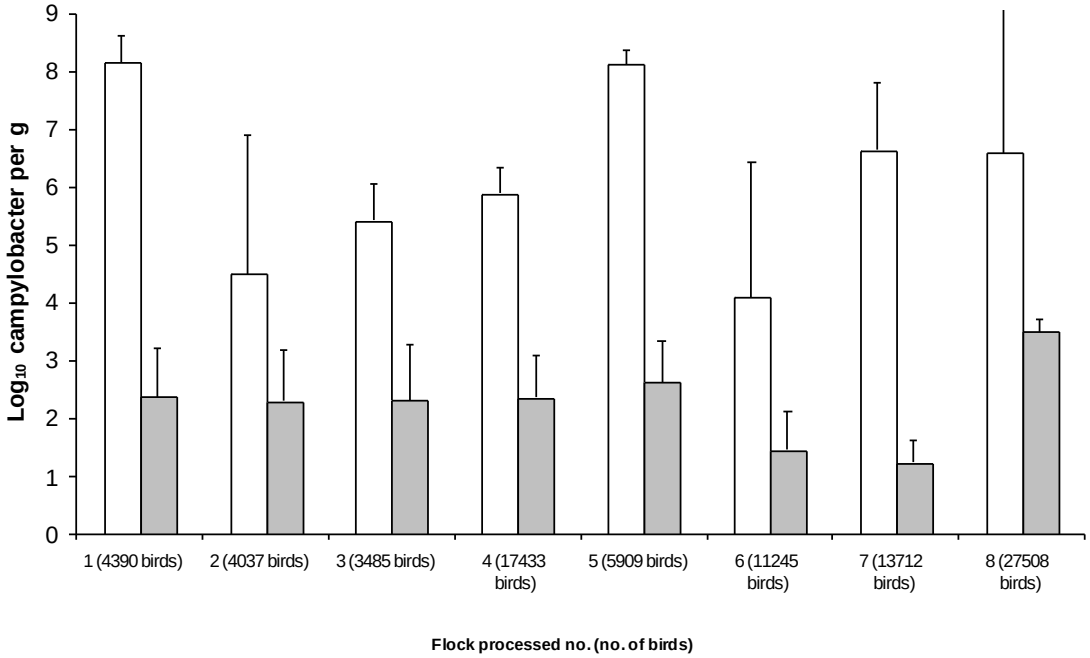

Figure 7. Levels of contamination in neck skin samples after final wash (grey shaded bars) in relation to levels in caecal contents (open bars) in the flock itself and in previous flocks. The flocks were processed at Plant 1 on one day (Day 1 August 2006). Error bars are SD.

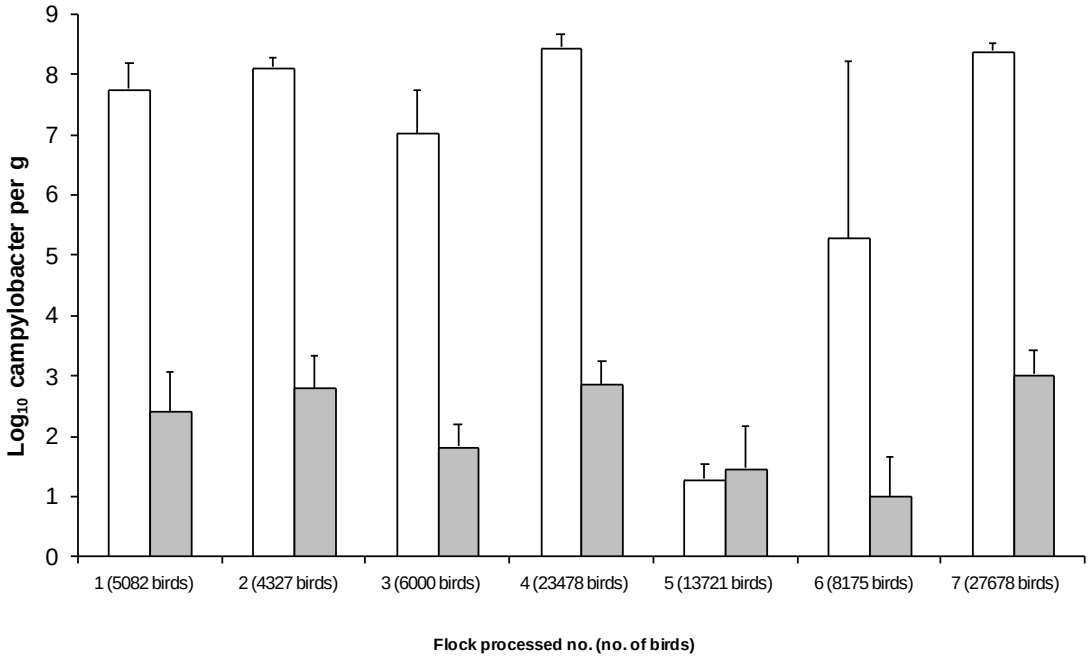

Figure 8. Levels of contamination in neck skin samples after final wash (grey shaded bars) in relation to levels in caecal contents (open bars) in the flock itself and in previous

flocks. The flocks were processed at Plant 1 on one day (Day 2 August 2006). Error bars are SD.

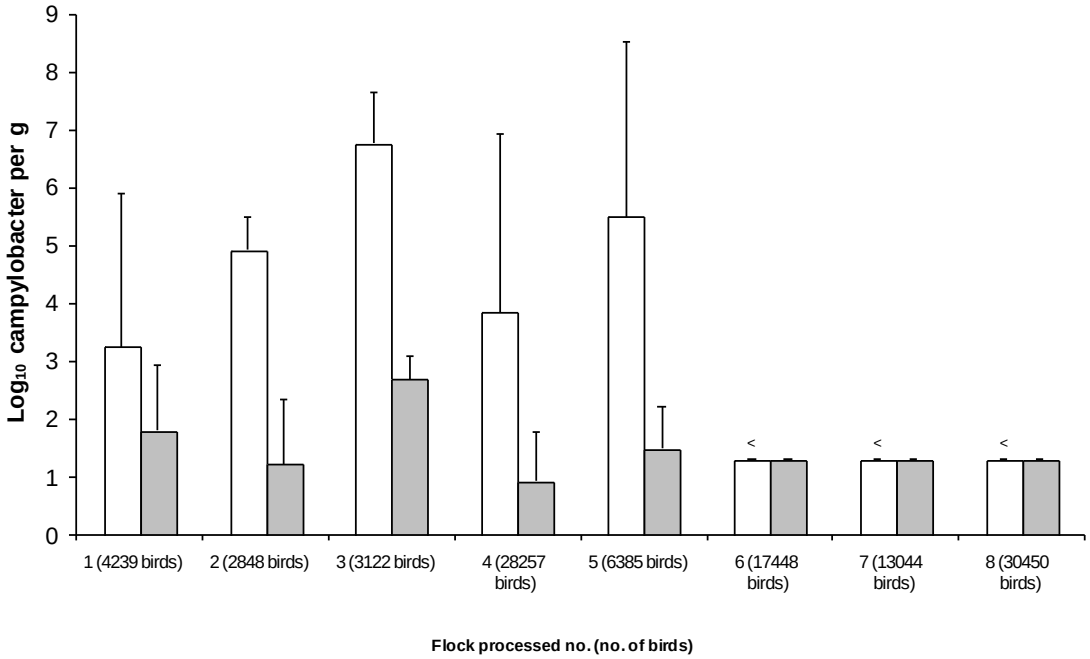

Figure 9. Levels of contamination in neck skin samples after final wash (grey shaded bars) in relation to levels in caecal contents (open bars) in the flock itself and in previous flocks. The flocks were processed at Plant 1 on one day (Day 1 September 2006). Error bars are SD.

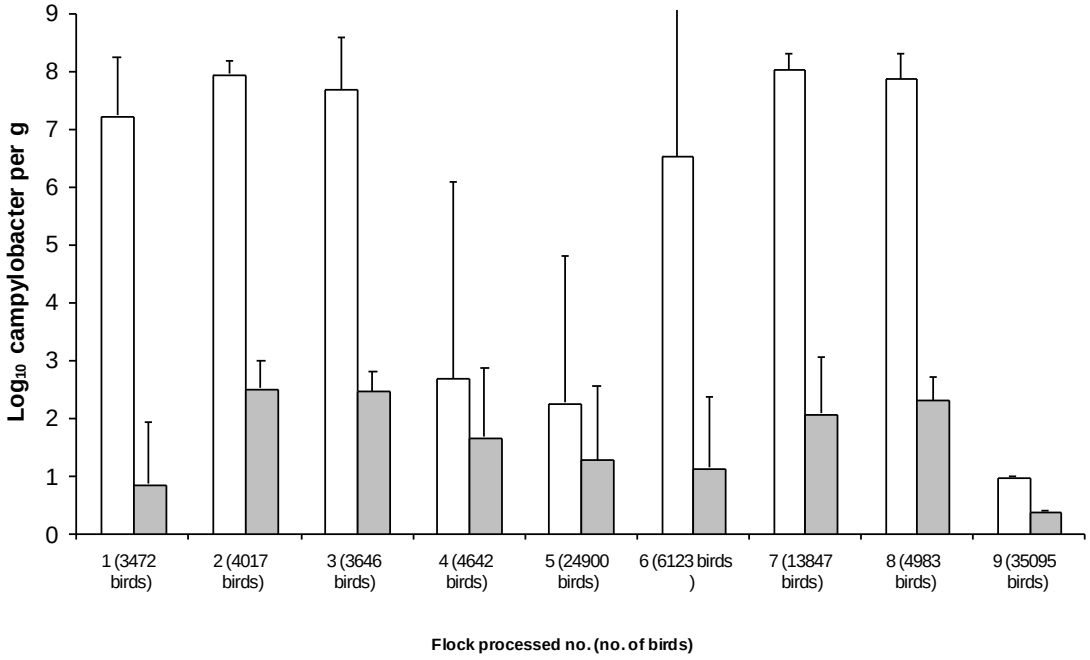

Figure 10. Levels of contamination in neck skin samples after final wash (grey shaded bars) in relation to levels in caecal contents (open bars) in the flock itself and in previous flocks. The flocks were processed at Plant 1 on one day (Day 2 September 2006). Error bars are SD.

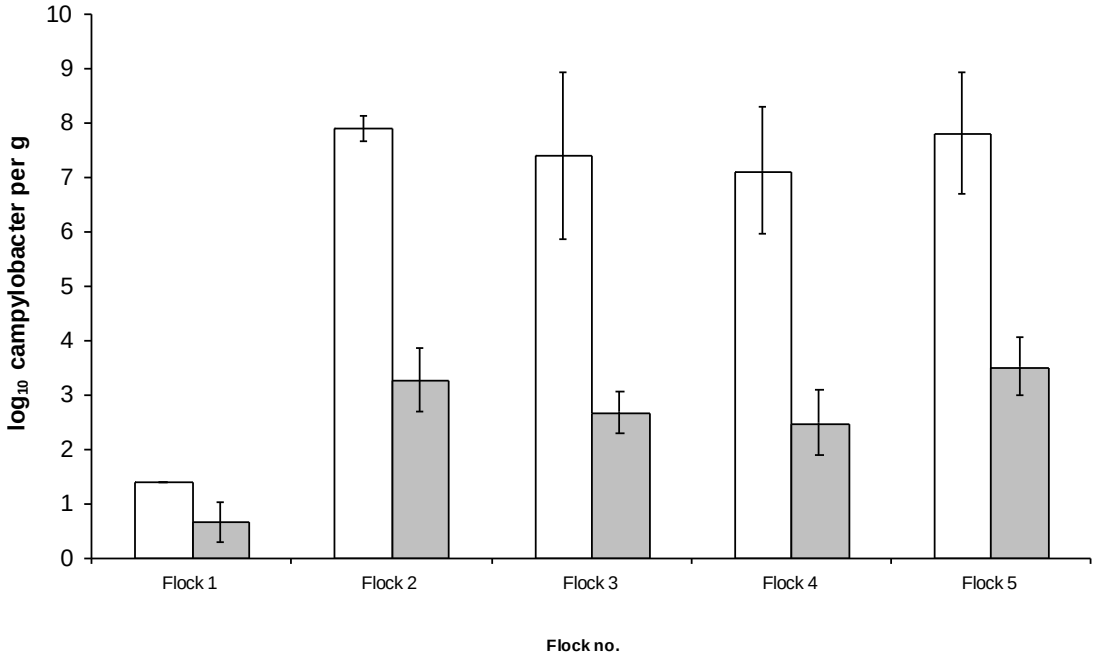

Figure 11. Levels of contamination in neck skin samples after final wash (grey shaded bars) in relation to levels in caecal contents (open bars) in the flock itself and in previous flocks. The flocks were processed at Plant 2 on one day (Day 1 July 2007).

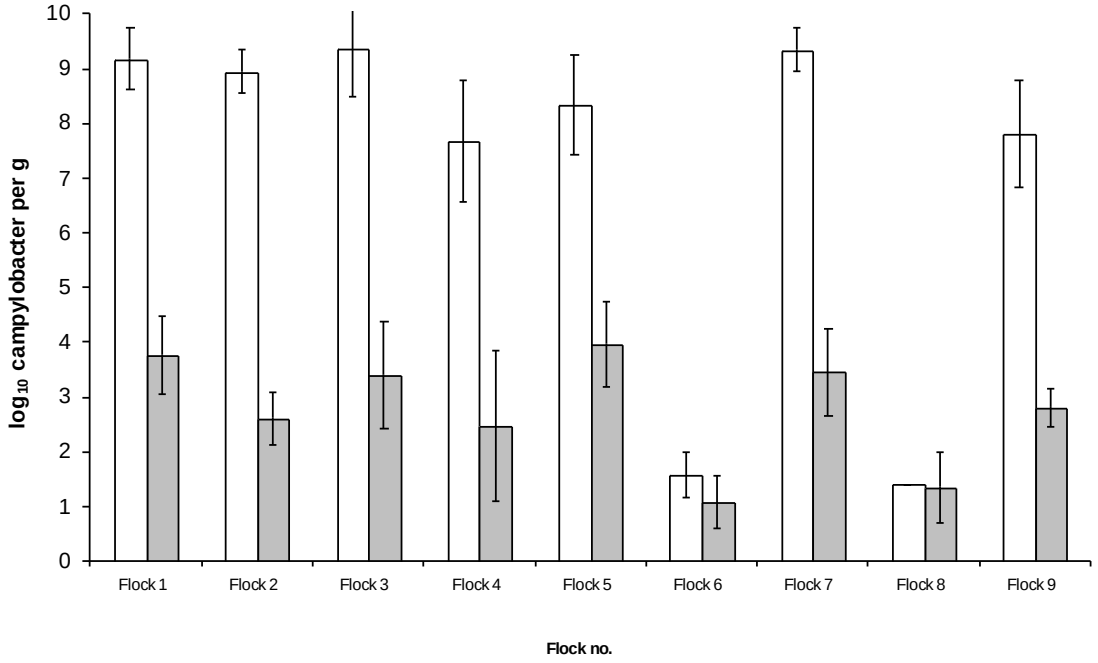

Figure 12. Levels of contamination in neck skin samples after final wash (grey shaded bars) in relation to levels in caecal contents (open bars) in the flock itself and in previous flocks. The flocks were processed at Plant 2 on one day (Day 2 July 2007).

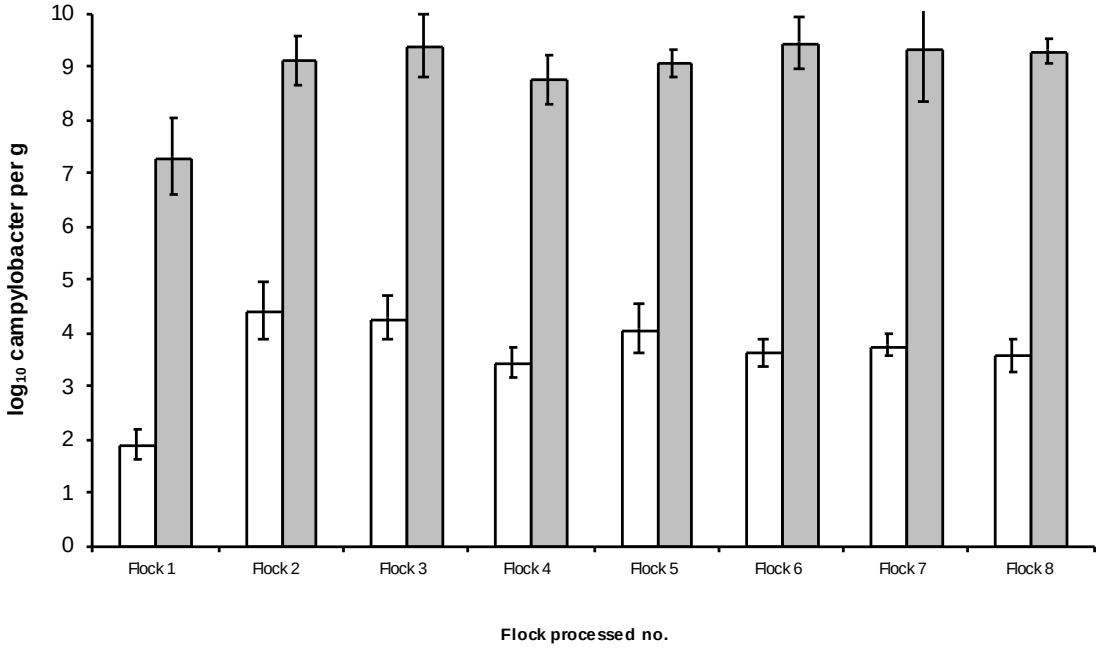

Figure 13. Levels of contamination in neck skin samples after final wash (grey shaded bars) in relation to levels in caecal contents (open bars) in the flock itself and in previous flocks. The flocks were processed at Plant 3 on one day (August 2007).

8.2.2 Investigation of contamination levels along the processing line

The levels of Campylobacters and Enterobacteriaceae contamination at five or six processing stages were investigated for the first four flocks of the day on four occasions (twice at Plant 1 and twice at Plant 2). Contamination was detected from after scald and in 3 of 4 flocks examined in Plant 1 on that day, and the numbers remained at this level at the subsequent processing stages (Figure 14 and Figure 15).

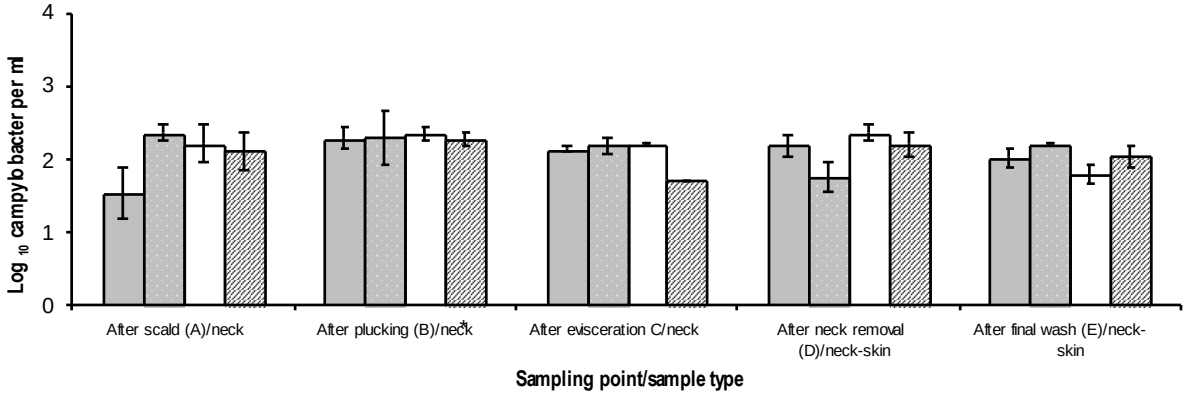

Figure 14. Numbers of Campylobacter in samples collected from carcasses at different stages of processing of four flocks processed on the same day in Plant 1. Every sample was suspended in 50 ml volumes and numbers are per ml of this volume. Four consecutive flocks were sampled with caecal contents levels of 8.4 $\log_{10}$, SD= 0.5 (grey shaded bar), 6.9, SD= 0.8 (dotted bar); 8.2, SD = 0.5 (open bar); and 8.3 SD = 0.4 (striped bar).

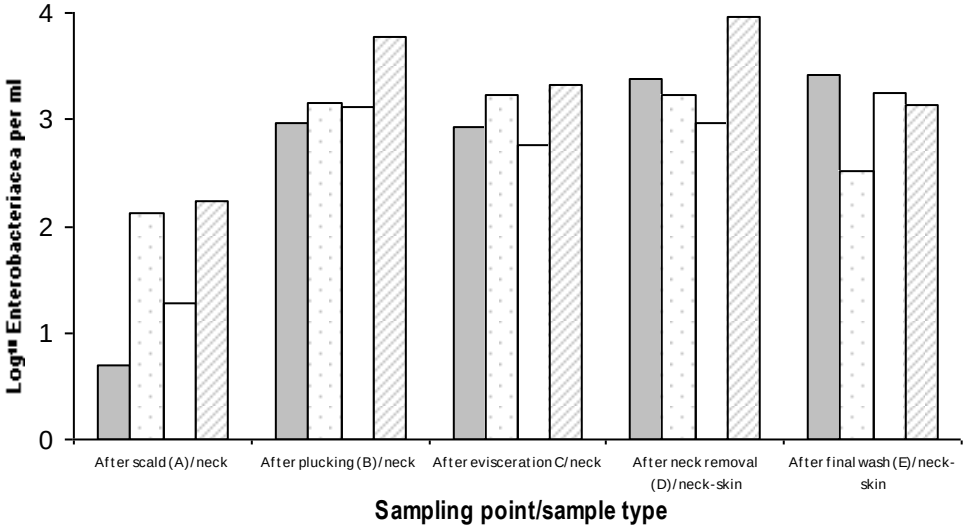

Figure 15. Numbers of Enterobacteriaceae in samples collected from carcasses at different stages of processing of four flocks processed on the same day in Plant 1. Every sample was suspended in 50 ml volumes and numbers are per ml of this volume. Four consecutive flocks were sampled with Campylobacter caecal contents levels of 8.4 log_{10}, SD= 0.5 (grey shaded bar), 6.9, SD= 0.8 (dotted bar); 8.2, SD = 0.5 (open bar); and 8.3 SD = 0.4 (striped bar). The flocks were the same as those in Figure 14.

In two flocks with high levels of campylobacters in caecal contents processed on another visit to Plant 1 numbers of campylobacters increased after scald but did not change markedly thereafter (Figure 16 and Figure 17).

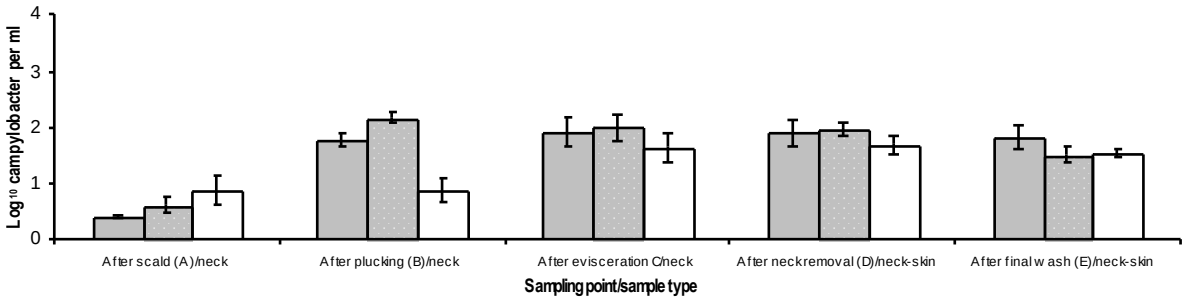

Figure 16. Numbers of Campylobacter in samples collected from carcasses at different stages of processing of three flocks processed on the same day in Plant 1. . Every sample was suspended in 50 ml volumes and numbers are per ml of this volume. The average number of campylobacters in caecal contents of the flocks processed were: 8.9 log_{10}, SD = 1.1 (grey shaded bar); 8.9, SD = 0.7 (dotted bar) and 6.2, SD = 2.8 (open bar).

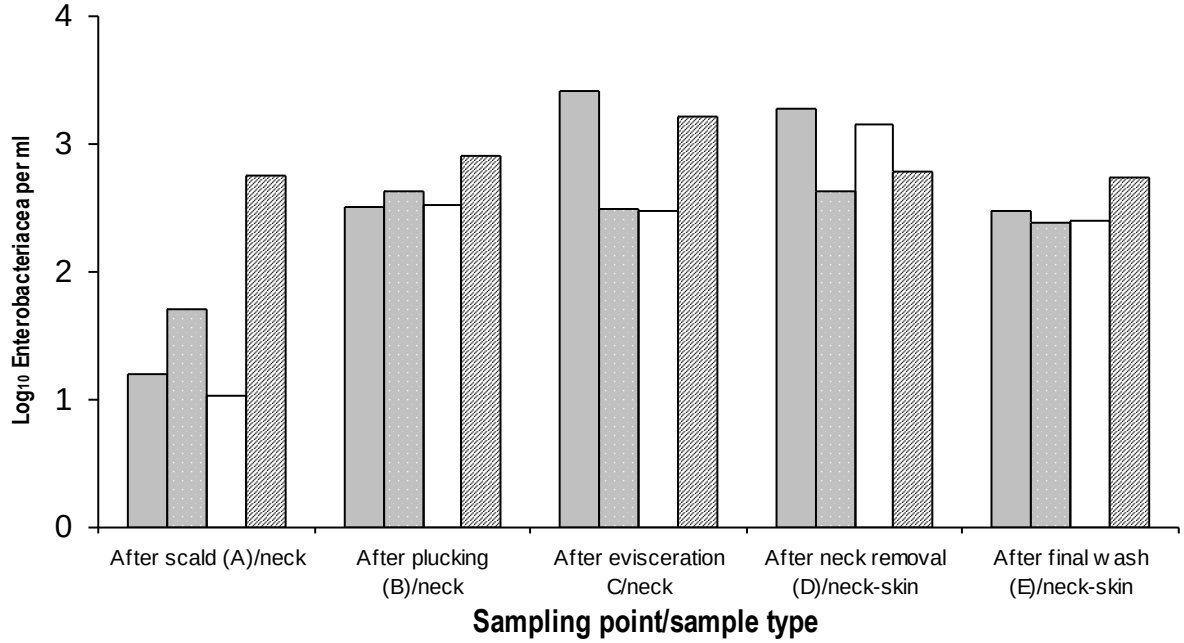

Figure 17. Numbers of Enterobacteriaceae in samples collected from carcasses at different stages of processing of four flocks processed on the same day in Plant 1. Every sample was suspended in 50 ml volumes and numbers are per ml of this volume. The average number of Enterobacteriaceae in caecal contents of the flocks processed were: $\log_{10}$ 7.6, SD = 0.6 (grey shaded bar); 7.9, SD = 0.2 (dotted bar); 7.1, SD = 0.6 (open bar) and 6.6 SD = 0.7 (striped bar). The flocks were the same as those in Figure 16.

Numbers in samples collected at processing points in Plant 2 were similar for four flocks processed consecutively. Overall, numbers were lower for two flocks after chilling but not for the third flock processed (Figure 18 and Figure 19).

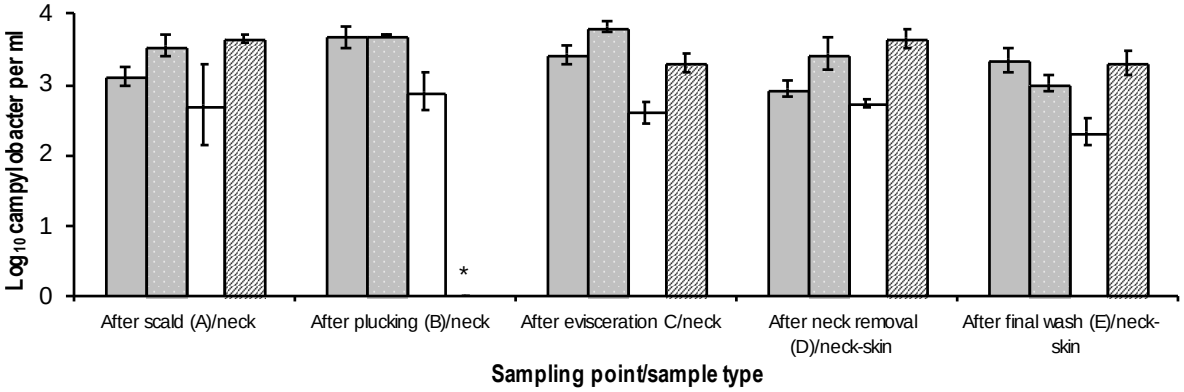

Figure 18. Numbers of Campylobacter in samples collected from carcasses at different stages of processing of four flocks processed on the same day in Plant 2. Every sample was suspended in 50 ml volumes and numbers are per ml of this volume. The average number of campylobacters in caecal contents of the flocks processed were: $\log_{10}$ 8.8, SD = 0.8 (grey shaded

bar); 9.2, SD = 0.5 (dotted bar); 8.7, SD = 0.5 (open bar) and 9.1, SD = 0.6 (striped bar). * data not obtained.

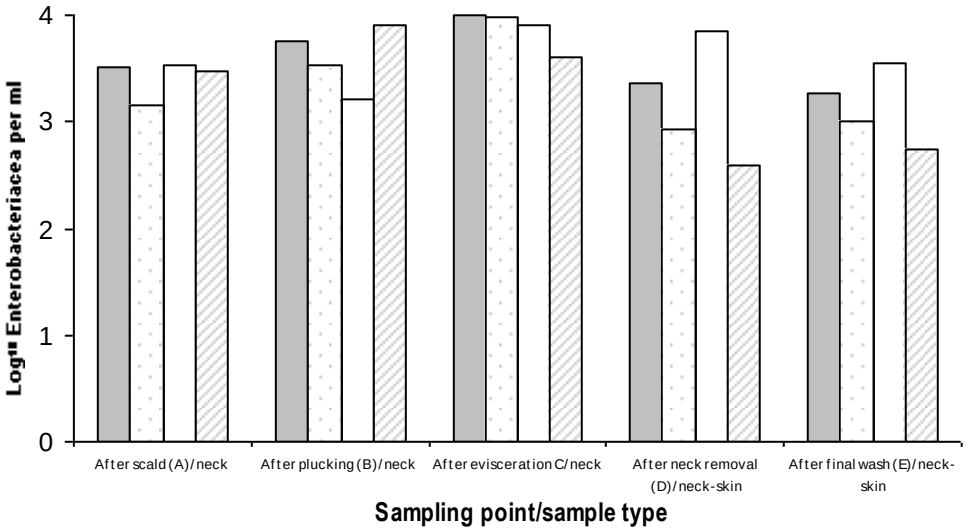

Figure 19. Numbers of Enterobacteriaceae in samples collected from carcasses at different stages of processing of four flocks processed on the same day in Plant 2. Every sample was suspended in 50 ml volumes and numbers are per ml of this volume. The average number of enteros in caecal contents of the flocks processed were: log_{10} 7.2, SD = 1.0 (grey shaded bar); 7.7, SD = 1.2 (dotted bar); 7.6, SD = 0.9 (open bar) and 7.9, SD = 1.1 (striped bar). The flocks were the same as those in Figure 18.

One flock studied on a second visit to Plant 2 also showed increased counts after plucking but little change in numbers thereafter (Figure 20).

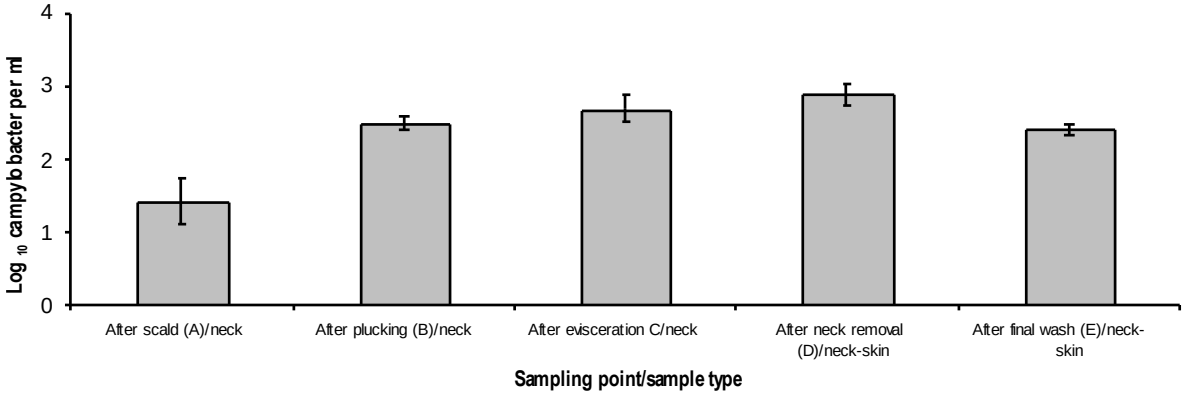

Figure 20. Numbers of Campylobacter in samples collected from carcasses at different stages of processing of a single flock. Every sample was suspended in fifty ml volumes

and numbers are per ml of this volume. No caecal contents were obtained from this flock. The flock was processed at Plant 2.

8.2.3 Investigation of Campylobacter contamination between plucking and final wash

The level of contamination between plucking and evisceration stages of processing was investigated in three experiments (Figure 21 to Figure 23). The level of Campylobacters was compared in samples from carcasses either heat treated immediately after pluck and rehung onto the processing line or left on the line through processing as usual.

In one flock investigated with caecal contents levels of 6.5 $\log_{10}$ per gram the mean $\log_{10}$ number of Campylobacters was 1.8 $\log_{10}$ in neck skin samples taken just after the final wash from carcasses which were heat-treated just after pluck compared to 2.6 $\log_{10}$ in those not heat-treated, i.e. left on the line (Figure 21).

Another flock investigated had caecal contents levels of 8.5 $\log_{10}$ and there was no significant difference in the level of Campylobacters between samples from heat-treated and not heat-treated carcasses (Figure 22). The $\log_{10}$ number of Campylobacters per ml in neck-samples collected just after plucking (point B) from heat treated carcasses was 0.7 (SD = 0.4). This was significantly ($p < 0.001$; students t-test) lower than in neck skin samples from heat-treated carcasses collected after the final wash stage (point E). A similar result was obtained for breast skin samples where the $\log_{10}$ number of Campylobacters per ml sample collected just after plucking (point B) from heat treated carcasses was 0.4 (SD = < 0.1). This was significantly ($p < 0.001$; Student's t-test) lower than in breast skin samples from heat-treated carcasses collected after the final wash stage (point E).

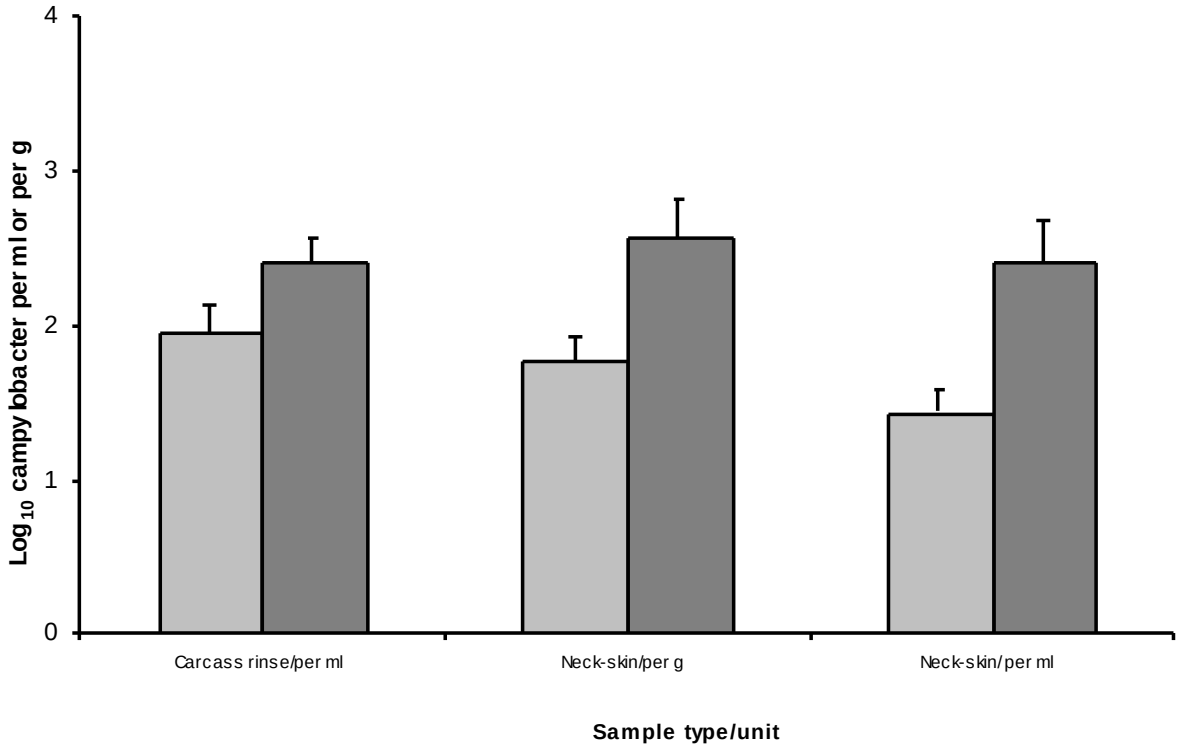

Figure 21. Numbers of Campylobacter after the final wash (point E) in samples from carcasses either heat-treated immediately after pluck (light grey bars) or not treated

(left on the line: dark grey bars). Error bars are SEM. The heat-treatment consisted of 2 min at 80°C. The average number of Campylobacter per g caecal content for the flock processed was $\log_{10}$ 6.5 (SD = 0.7). The flock was processed at Plant 1.

Also in a third flock investigated with caecal contents levels of 9.3 $\log_{10}$ there was no significant difference in the level of Campylobacters between samples from heat treated and non-heat treated carcasses taken just after evisceration (Figure 23). The heat treatment resulted in a significant ($p < 0.007$; Student's t-test) difference in the level of Campylobacters in neck samples examined immediately after plucking. There was a significant increase in the numbers of Campylobacters between after plucking compared to after evisceration/after final wash in samples from heat treated carcasses.

8.3 Discussion

The contamination of carcasses was related to the level in caeca, although it is possible that factors including those related to operative procedures in plants affect levels of contamination at different stages. Contamination was detected from after scald and increased on two of four occasions tested after plucking. There was little evidence for any change in contamination levels during subsequent stages of processing. In order to quantify better the extent of contamination between plucking and evisceration, carcasses, which had been heat-treated to eliminate the majority of contamination up until immediately after plucking, were rehung onto the line and examined after evisceration (point C) and after final wash (point E). These experiments demonstrated that a considerable amount of contamination was taking place after plucking and until just after the final wash (point E). It was also demonstrated in one trial that the evisceration process itself contributed a considerable amount of this contamination (Figure 22). This suggested that intervention during processing in stages before plucking is unlikely to reduce the final level of carcass contamination as there is additional considerable contamination after plucking, at evisceration, and up to before the final wash.

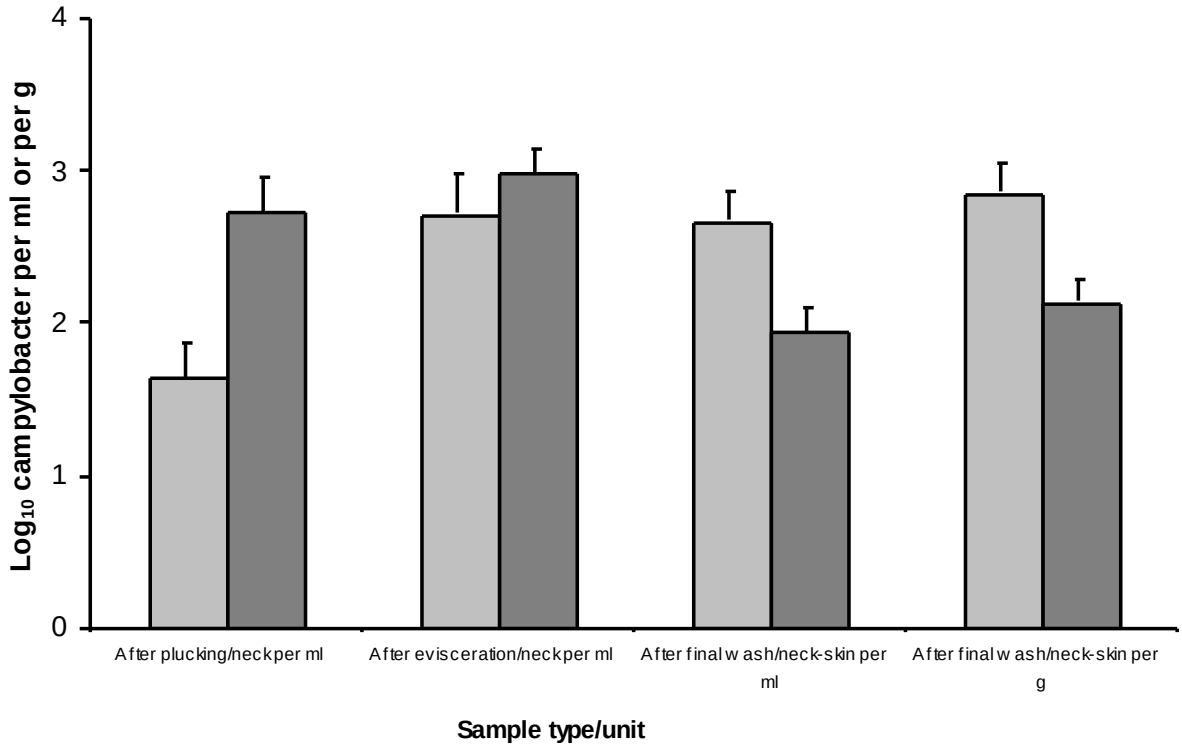

Figure 22. Numbers of Campylobacter in samples from either carcasses heat-treated immediately after pluck carcasses (light grey bars) and rehung onto the processing line (light grey bars at points C and E) or from carcasses not treated/left on the line (dark grey bars). Error bars are SEM. The heat-treatment consisted of 2 minutes at 80°C. The average number of Campylobacter per g caecal content for the flock processed was 9.3 $\log_{10}$ (SD = 0.6). The flock was processed at Plant 1.

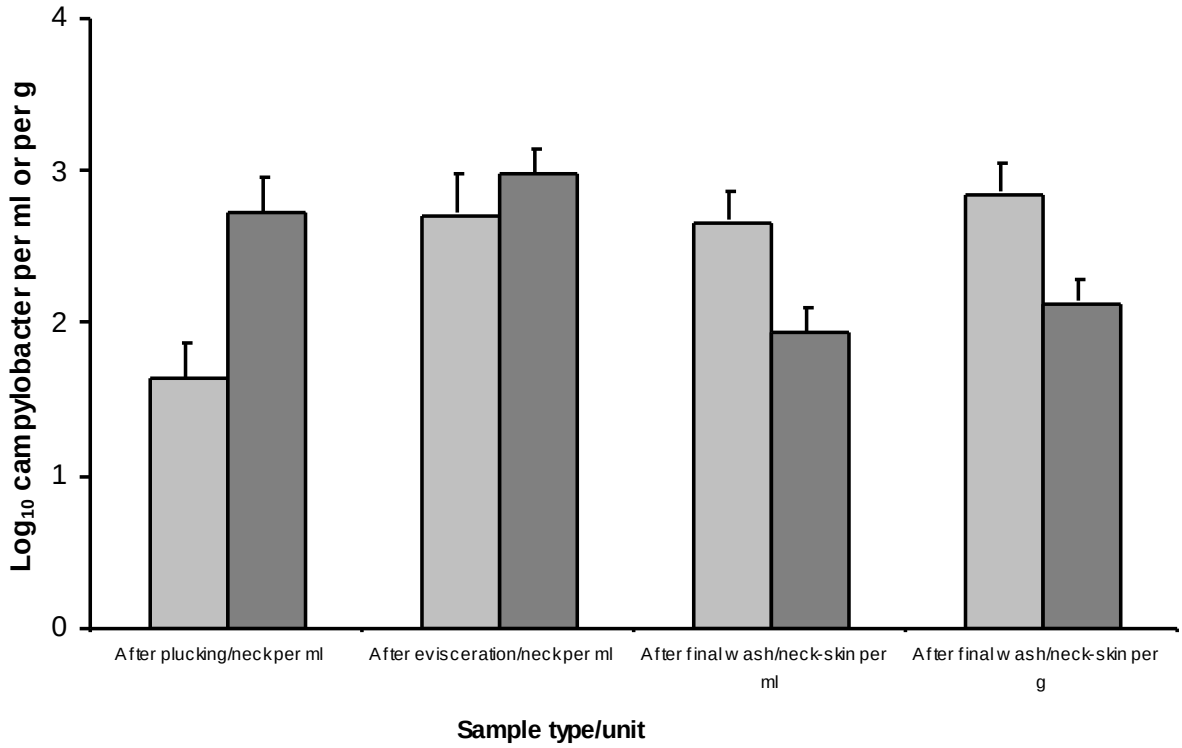

Figure 23. Numbers of Campylobacter in samples from either carcasses heat-treated immediately after pluck carcasses (light grey bars) and rehung onto the processing line (light grey bars at points C and E) or from carcasses not treated/left on the line (dark grey bars). Error bars are SEM. The heat-treatment consisted of 2 minutes at 80°C. The average number of Campylobacter per g caecal content for the flock processed was 9.3 $\log_{10}$ (SD = 0.6). The flock was processed at Plant 1.

8.4 Conclusions

The level of Campylobacters in caecal contents was associated with the level found in neck skin samples but this relationship was also influenced by plant operational procedures. The number of Campylobacters on carcasses from Campylobacter-negative flocks, processed immediately after positive flocks, were low, even when several positive flocks had gone through the plant.

During normal processing, there is little evidence for significant differences in the level of Campylobacter contamination measured after plucking to after the final wash.

Both plucking and evisceration processes contributed significantly to contamination of carcasses as demonstrated by examining contamination of heat-treated carcasses placed in the processing line just before those stages. The later stages were therefore considered to be more effective intervention points. The number of Campylobacters on carcasses just before chill were compared to levels of Campylobacters in caeca in three processing plants. There was a significant relationship between the numbers in caeca and those found on carcasses.

9 Ranked assessment of alternative/novel methods/processes

From the various literature studies, site visits and discussions within the project team, a number of possible measures were identified to reduce Campylobacter contamination of chicken carcasses. The possible study areas split into two broad categories: new intervention processes and investigations focussed on changes to current processes or equipment.

9.1 New Interventions

9.1.1 Location

New interventions would be additional processes added to the current production line(s). A vital task is to identify the best location along the line for this/these additional process(es). When considering locations for new processes, possible treatment times, footprint of equipment and space in factory need to be considered in combination with the microbial efficacy of any treatment.

The four most likely locations are:

Post pluck – many studies have identified the plucker as a major contamination point. Interventions located here would be able to take effect before bacteria from the plucker had fully attached. There is generally some space in most plants after the plucker, as the lines move into a separate area for evisceration

Post evisceration (EV) – as with pluckers, early action is appropriate to minimise time available for attachment and reduce the risk of cross-contamination to other carcasses. There is generally some space in most plants post EV at and around the point where carcasses and their viscera are inspected.

Post Inside/Outside (I/O) wash – the I/O wash is the last process before chilling. There is little subsequent risk of contamination with Campylobacters and thus carcasses treated here should remain clean. There is also the potentially long treatment time as carcasses pass through the air chilling process that follows the I/O wash. There is usually plenty of line length to install interventions between the I/O wash and chiller entry.

Post chill – this is the last possible position and is thus closest to the point of consumption of the meat. There is commonly plenty of line length available at the chiller exit.

9.1.2 Treatment

Physical and chemical treatments are possible. A key difficulty with all these methods is the interaction between the functional parameters of the application method i.e. for a spray (pressure, flow rate, droplet size, etc.) or for immersion (agitation and flow in tank, speed of traverse, transfer times, etc), and decontamination effect.

9.1.2.1 Physical:

The prime physical mechanism for decontamination is through heat based inactivation of bacteria. This heat could be applied in a number of ways:

a. Hot water spray

b. Hot water immersion

c. Atmospheric steam; including the ‘Sonosteam’ process with ultrasonic excitation of steam nozzles.

d. Gas flame singeing

e. Dry heating

Non-heat based physical methods are:

f. High impact/volume cold water spray

g. Exposure to ultraviolet (UV) light

h. Exposure to microwave energy

i. Spray washes with ultrasonic excitation of nozzles.

9.1.2.2 Chemical:

Chemical decontamination treatments by immersion or spraying with aqueous solutions of the following chemicals are worthy of investigation.

a. Chlorine (Cl)

b. Chlorine dioxide (ClO_2)

c. Acidified sodium chlorite (ASC)

d. Tri-sodium phosphate (TSP)

e. Peroxy acetic acids (PAA)

f. Ozone (O_3)

g. Electrolysed water

9.2 Process or Equipment Measures

Changes to existing processes or equipment may also have the potential to reduce Campylobacter in chickens. The selection of appropriate areas will be informed by the results of the study to identify the contamination routes (Objective 02).

9.2.1 Major developments or changes

9.2.1.1 Remove skin with feathers attached

Scalding and plucking are considered by many to present potential cross-contamination risks due to passing every carcass through a "scald" tank (at 52-54°C) containing a 'soup' of faecal matter, feather and other debris, then passing it through a complex and difficult-to-clean plucking machine. It is possible to skin a chicken with the feathers still in place and much poultry meat is sold skinless. The reduction in numbers of Campylobacters achieved by skinning rather than plucking chicken carcasses could be evaluated and if beneficial, entirely new equipment to replace scalder and plucker could be developed.

9.2.1.2 Drying tunnels

There is a body of thought that suggests surface drying inactivates Campylobacter. Incorporation of drying tunnels using warm air and/or air knife technology to keep chickens surfaces dry may have some benefit.

9.2.1.3 Cloacal plugging

Studies suggest that faeces are squeezed out from the vent/viscera during processing. This then adds to the contamination issues. Some studies have shown that suturing the vent or introducing acid into the cloaca reduces numbers of faecal bacteria on the carcass. These techniques could be evaluated and methods of cloacal plugging of each bird at line speeds could be developed. This should reduce contaminants in scalder, plucker and evisceration process until the viscera are inspected.

9.2.1.4 Ultrasonic aided scald & defeather

Ultrasonic baths are for cleaning of mechanical parts with the vibrations aiding the dislodging of physical debris from the surface of the part. It could be the case that scalding in an ultrasonic bath aids microbial detachment and feather loosening.

9.2.1.5 Steam scalding

The tank scalder 'soup' is seen by many as a potential contaminator of the whole poultry production process. Furthermore, it is probable that dirty and/or contaminated scalder water trapped in the feathers is carried from the scalder to the plucker, exacerbating contamination and cross-contamination problems in the plucker. Some studies suggest that steam or hot moist air scalding systems would improve hygiene over typical tank based systems. These claims could be evaluated in detail for Campylobacter.

9.2.1.6 Plucking

9.2.1.7 Air flow control

Some studies indicate substantial airborne cross-contamination in the plucker room. By enclosing plucker equipment and controlling and filtering the airflows, this potential cross-contamination route could be reduced. Air curtains and/or physical screens could be investigated to confine airborne detritus to the plucker area. Alternatively, a U–shaped deviation in the line could be constructed, enclosed by a close-fitting tunnel through which birds travel, with extraction at base of the 'U' that pulls air from plucker and clean zones thus preventing air transfer from plucker zone to the cleaner downstream processes.

9.2.1.8 Improved post-pluck wash

All plants have a carcass rinse/wash after plucking to help remove remaining physical debris. Improvements to design of this current cold spray washing system could be investigated. Improvements could include; increasing washing efficacy replacing the currently used cold water, using heated water, or adding chemicals to the rinse for antibacterial effect, or a combination of these possibilities.

9.2.1.9 Waste flume

Waste feathers are typically removed from the plucker zone by a water flume, typically feathers are filtered out and the water is recirculated. The microbiological state of this spray-generating turbulent flow probably contributes to bacterial levels in the plucker area. Antimicrobial treatment of flume water before recirculation could be addressed to reduce bacterial level on chickens.

9.2.1.10 Adaptable evisceration equipment

Viscera can be ruptured in the evisceration equipment so that intestinal contents are subsequently spread onto the carcass. Improving the design of the EV equipment, especially to adapt to the changes in the size and shape of each carcass so as not to rupture the viscera could reduce Campylobacter contamination.

9.2.1.11 Flail wash

Some plants have an additional 'flail wash' before the I/O washer. The flail washer provides a mechanical washing action with sets of rotary brushes and flails operating within a spray cabinet. The efficacy of this unit could be evaluated to determine whether the process should be recommended to all poultry producers.

9.2.1.12 Chicken line 'pigging'

Most EV equipment is complex and difficult to clean because of poor accessibility to internal functional parts. If cross-contamination via machinery routes is shown to be important, development of a 'pigging' system, similar to that used to clean inside pipelines could be developed. The chicken line pig would consist of several modules the size of chicken carcasses hung on the line applying detergents, brushes, rinses, disinfectants, etc. As the line was run the pig would clean inside the machine, cleansing the difficult to reach parts that contact the chickens and transfer cross-contamination.

9.2.1.13 Continuous equipment surface cleaning

If cross contamination contact points can be identified, it may be possible to develop continuous surface cleaning technologies such as flowing detergents over the surface, wipers to remove detritus, etc.

9.2.1.14 Remove cross contamination points

Cross-contamination of bacteria can be caused by every carcass rubbing against the same parts of equipment or when carcasses touch each other during processing. It may be possible to redesign processes to remove rubbing contacts, and avoid bulking carcasses together and thus reduce Campylobacter cross-contamination.

9.2.2 Minor changes to existing processes.

9.2.2.1 Wash water additives

Many pieces of EV equipment incorporate water sprays to clean equipment, lubricate the process and clean the carcasses. The inclusion of chemicals or hot water might reduce Campylobacter contamination. Possible chemical and heat based approaches are listed in section 9.1.2.

9.2.2.2 Scheduling

If the Campylobacter status of each flock is known by producers before slaughter, C+ flocks could be scheduled to be processed after C– flocks; this should reduce the likelihood of cross-contamination.

9.2.2.3 Plucker fingers design and materials improvement

The rubber plucker fingers are in contact with every carcass as it passes through the process and are a potential cross-contamination route. Many rubber fingers exhibit small surface cracks caused by their continual flexing and these crevices could easily harbour Campylobacter and other unwanted organisms, increasing the risk. The effect and influence of plucker finger design and replacement strategy on cross contamination could be further evaluated. Plucker finger redesign could result and be a readily retrofit-able measure.

9.2.2.4 . Pre scald feet cleaning

Removal of gross debris from the feet before scalder entry (as seen in one turkey plant) may warrant investigation for chicken.

9.2.2.5 Improve existing sprays

There are many places in the current processing line where water sprays are used for machine or carcass cleaning and process lubrication. Many of these are poorly designed and ineffective. Development of spray testing regimes, engineering analysis and design/operation improvements for these existing sprays may yield carcass microbial benefits.

9.3 Initial evaluation and selection of possible approaches

The possibilities were considered by the project team and divided into high, medium, and low priorities for research activities within this M01039 project. The categories were assigned on the basis of expected effect versus required development or implementation difficulties.

Higher priorities were generally assigned to intervention methods that could readily be retro-fitted or flexibly applied in many locations along the production process giving more likelihood of overall success.

Possible approaches requiring major changes to the processing line will not be readily applicable by the poultry industry and were generally considered low priority for these investigations. Similarly, approaches that require substantial developments were also considered low priority as there is a risk in whether the concept is effective and longer development time scales to implementation.

Objective 02 will identify the prime Campylobacter contamination and cross-contamination routes. These results will determine the most effective location for a new decontamination intervention.

9.3.1 High priority investigation areas

These approaches/concepts will be addressed first in this project.

Concept/Approach	*Comment*
Acidified sodium chlorite (ASC) spray	Flexible concept applicable as new decontamination intervention stage or as additives to existing equipment washes/sprays. On EFSA shortlist of possibly permitted additives
Chlorine (Cl) spray	Flexible concept applicable as new decontamination intervention stage or as additives to existing equipment washes/sprays.

Chlorine dioxide (ClO_2) spray	Flexible concept applicable as new decontamination intervention stage or as additives to existing equipment washes/sprays. On EFSA shortlist of possibly permitted additives
Peroxyacetic acids (PAA) spray	Flexible concept applicable as new decontamination intervention stage or as additives to existing equipment washes/sprays. On EFSA shortlist of possibly permitted additives
High impact/volume cold water sprays	Relatively easy change to existing process sprays
Hot water spray	Easy change to existing process sprays
Trisodium phosphate (TSP) spray	Flexible concept applicable as new decontamination intervention stage or as additives to existing equipment washes/sprays. On EFSA shortlist of possibly permitted additives

9.3.2 Medium priority investigation areas

These will be investigated if possible, opportunity, time and resources permitting. If not addressed, future projects should at least conduct initial studies for basic assessment of feasibility.

Concept/Approach	*Comment*
Atmospheric steam immersion	Simple concept but space needed to fit into existing lines
Chicken line 'pigging'	Substantial developments required, but potential to make major impact on efficacy of machinery cleaning. Re-assess in light of objective 02 results.
Cloacal plugging	Scientific evidence to support concept but significant engineering development required to implement at line speeds
Dry heating	Simple concept but space needed to fit into existing lines
Electrolysed water	Easy integration with existing processes, but not currently used or on EFSA shortlist
Flail wash	Existing equipment used in some plants. If efficacious can readily be utilised with no technology transfer risk.
Gas flame singeing	Simple intervention technology transfer from pork industry
Hot water immersion	Simple concept but space needed to fit into existing lines
Improve existing sprays	Needs a plant by plant approach. Long term and extensive effort required.
Improved post-pluck wash	Can be addressed by high priority additives and washer design improvements

Ozone (O_3)	Easy integration with existing processes, but not currently used or on EFSA shortlist
Plucker finger design and materials improvement	Re-assess in light of Objective 02 results on routes of cross-contamination.
Plucking air flow control	Re-assess in light of Objective 02 results on routes of cross-contamination.
Scheduling	Re-assess in light of Objective 02 results on routes of cross-contamination.
Spray washes with ultrasonic excitation of nozzles	These mechanical changes not as straightforward as changes to sprayed solution
Steam scalding	Scientific evidence to support concept, but not readily applicable to existing plants currently with scald tank systems installed
Wash water additives	Easy implementation; use direct intervention studies to select appropriate for trials

9.3.3 Low priority investigation areas

These will not be investigated in this study, although future projects could provide initial studies for basic assessment of feasibility.

Concept/Approach	*Comment*
Adaptable evisceration equipment	Long development timescales, major factory refit required for implementation
Continuous equipment surface cleaning	Low expected benefit (re-assess in light of objective 02 results)
Drying tunnels	Low available time for exposure
Exposure to microwave energy	Low available time for exposure
Exposure to ultraviolet (UV) light	Difficulties of shadowing effects (especially in cavity), low available time for exposure
Plucker waste flume	Low expected benefit (re-assess in light of objective 02 results)
Pre scald feet cleaning	Low expected benefit (re-assess in light of objective 02 results)
Remove cross-contamination points	Long development timescales, major factory refit required for implementation (re-assess in light of objective 02 results)
Remove skin with feathers attached	Long development timescales, major factory refit required for implementation
Ultrasonic aided scald & defeather	Long development timescales, major factory refit required for implementation

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10 Evaluation of intervention methods

An evaluation of physical interventions was previously carried out during FSA Project M01019 (Physical Methods Readily Adapted to Existing Commercial Lines for Reducing Pathogens, Particularly Campylobacters, on Raw Poultry). Although that project reviewed scientific literature on chemical interventions, no practical evaluations were carried out. Experimental work in this project compared the effectiveness of a range of possible chemicals and physical methods for reducing numbers of Campylobacters on chicken carcasses.

10.1 Materials and methods

10.1.1 Equipment

Ideally, any intervention system that would be of interest to a poultry processor has to be able to be retrofitted to an existing line, close to the inside-outside washer, and should not require a large amount of space. In this project, experimental work centred on readily implementable spray systems and atmospheric steam treatment.

Five separate types of spray system were used, each with different characteristics for specific functions (Table 14).

Table 14. Spray system Characteristics

Spray type	Function	Characteristics
Washing	Impact jets to dislodge surface organisms, and sufficient flow to wash away debris	Full cone nozzles: large droplets, medium flow, medium pressure.
Misting	'Soft' spray to gently deposit active chemical onto surface	Flat fan nozzles, fine droplets, low flow, low pressure
Rinsing	Large volumes to remove chemical residues from surface	Full cone nozzles: large droplets, high flow, medium pressure.
Cooling	Reduce surface heat after steam treatment with cold water spray	Full cone nozzles: large droplets, high flow, low pressure.
High Intensity	High impact for mechanical dislodgment of detritus and organisms followed by deluge rinse for removal.	Flat fan, small droplets, medium flow, high pressure followed by Full cone nozzles: large droplets, high flow, medium pressure.

In most cases these spray configurations were implemented inside a purpose built spray rig consisting of an aluminium frame, with an overhead track to carry a shackle.

The shackle was driven along the track to replicate the moving line as seen in poultry production plants. The sides of the frame were enclosed with acrylic panels to contain over-spray. Various spray bars and nozzles were located within the enclosure for each configuration.

For cooling after steam treatment and for rinsing after a delay to allow chemicals to act, a separate static spray rig was used.

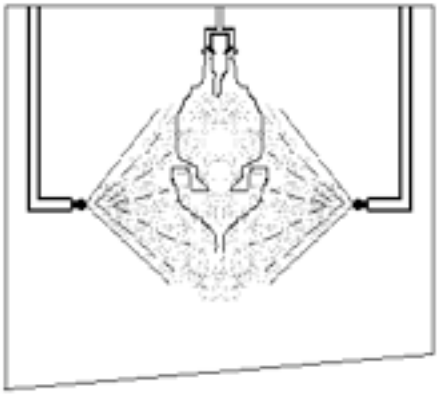
Each of the spray systems and the atmospheric steam unit are described in the following sections.

10.1.1.1 Washing spray system

The design aim of the washing spray system was to use large volumes at higher pressures to wash matter from the surface. The cabinet was configured with eight double nozzles on the

lateral sides of the chicken (Figure 24). A centrifugal pump (Ondina 100M, Sealand Ltd, Italy) generating a mean flow of 39.7 litres per minute supplied to the spray bars.

Overview of washing spray equipment



Detail of washing spray configuration

Figure 24. Washing spray configuration

10.1.1.2 Misting spray system

The design aim of the misting system was to produce small droplets at low pressure for application of chemicals in an even mist over the carcasses. Twelve 80º flat fan nozzles (8259150, Dual Pumps, UK) situated on spray bars to either side and below the carcasses (Figure 25) were supplied by a piston pump (D3735H 701 1 ARL, Flojet, UK) at a pressure of approximately 4 bars and a mean flow rate of 6.86 litres per minute.

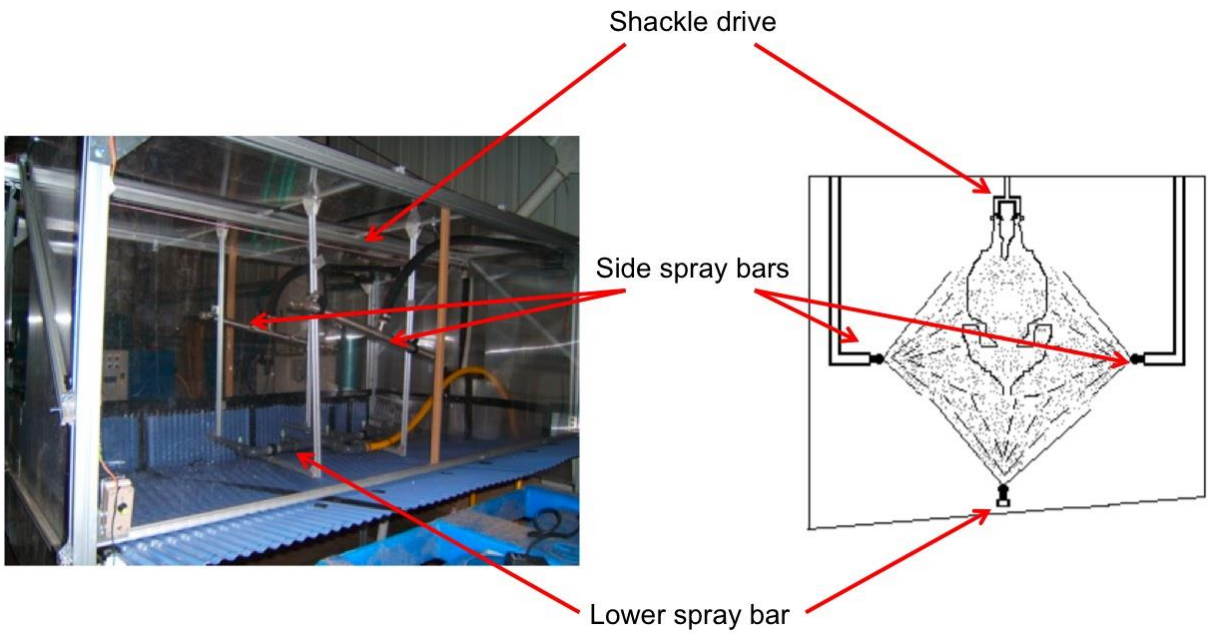


Figure 25. Misting spray configuration

10.1.1.3 Rinsing spray system

The design aim of the rinsing system was to rinse chemical residues from chicken carcasses after misting or spraying treatments. This system comprised two spray bars placed vertically on each side of the carcass (Figure 26). Each spray bar had two or three nozzles (type ¼.G.SS.10, Full Cone Jet, Spray systems, Illinois, USA) to spray the carcass. The carcass was placed between the jets using a mobile rig in which the carcass was suspended. The water was stored in a tank of about 125 litres capacity before being delivered to the rinsing spray bars by a centrifugal pump (Ondina 100M, Sea Land, Italy) with a mean flow rate of 35.7 litres per minute.

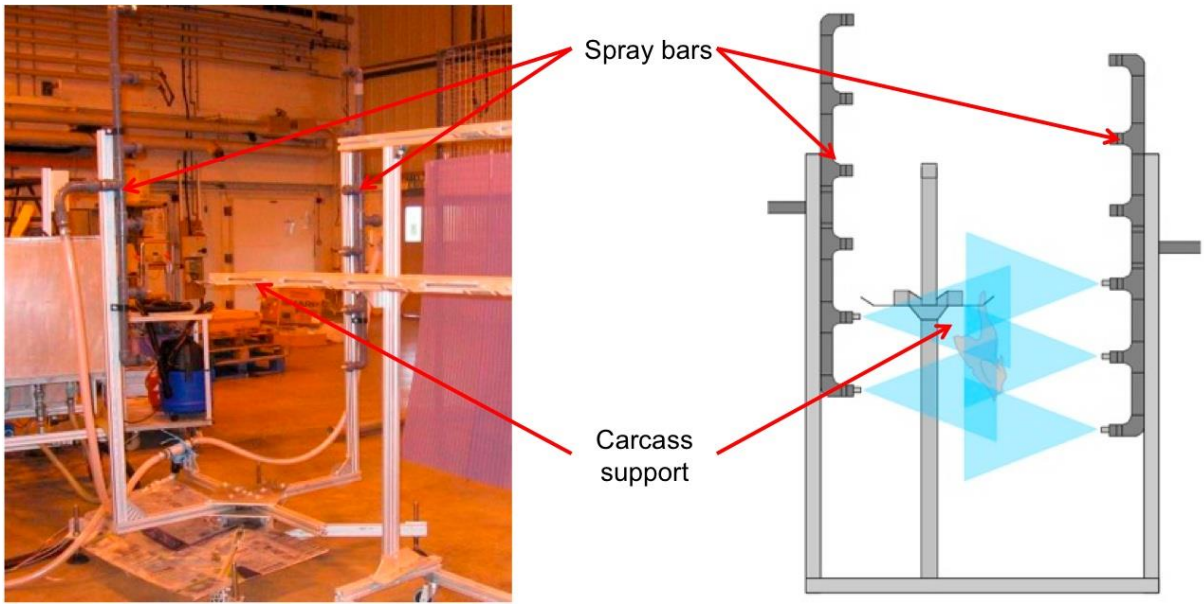


Figure 26. Rinsing spray system

10.1.1.4 Cooling spray system

The design aim of the cooling system was to reduce surface temperatures after steam treatments. This also provided a potentially beneficial washing effect. The cooling system was identical to the rinsing system albeit located such that the carcass was sprayed immediately after steam treatment.

10.1.1.5 High pressure and deluge cold water

The design aim of this system was to use high impact pressure jets to dislodge organisms and physical debris, followed by a deluge wash to rinse the detritus from the surface. Twin 15° flat fan nozzles (Spray Systems, Illinois, USA) each supplied by a separate piston pump (Challenge Pumps, UK) provided the initial impact jets. Eight full cone nozzles supplied by a centrifugal pump (Ondina 100M, Sea Land, Italy) provided the wash-off sprays.

10.1.1.6 Atmospheric steam

The steam system (Figure 27) comprised three 2.8 kW boilers that supplied steam at atmospheric pressure (*ca*. 100°C) to a plenum above an open based treatment chamber. A baffle plate evenly distributed steam into the main chamber. The front of the treatment chamber incorporated a double glazed panel to allow carcasses to be observed during treatment. Carcasses were lifted singly into the chamber on a gambrel attached to a time delay pneumatic

cylinder. The only variable in treatment was the time for which a carcass was raised into the chamber.

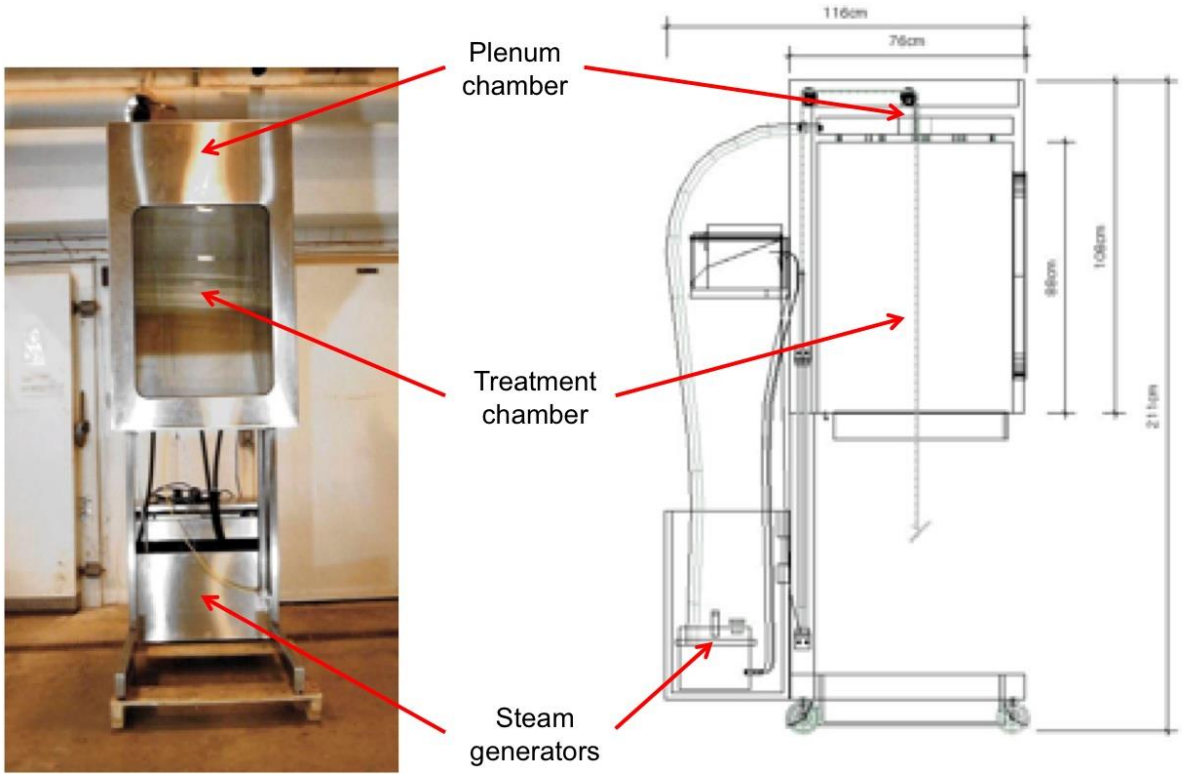


Figure 27. Atmospheric steam treatment cabinet

10.1.2 Chemical treatments

Aqueous solutions of chlorine dioxide (CD), chlorine (CL), acidified sodium chlorite (ASC), peroxyacetic acids (PAA), and trisodium phosphate (TSP) were investigated in this study. Additionally, hot water (HW), steam (stm) and ozonated water (Ozone) were used. Cold water (CW) was used as a control in chemical evaluations, for rinsing, for cooling and the high intensity spray configurations.

ASC, PAA and TSP treatments had been declared as of no safety concern by EFSA (2005b). Ozone is considered as acceptable in potable water according to the directive 98/83/EC and to the Drinking Water Inspectorate (2007), and thus can also be used in food industry to wash foods.

10.1.2.1 Acidified Sodium Chlorite (ASC)

The trials used an ASC based sanitising agent (Sanova, Ecolab). Sixty-litre batches of ASC solution were made with 475 ml of Sanova base (VR-2890-146, Sanova) diluted in approximately 30 litres of mains water. Then 1.44 litres of Sanova activator (VR-2890-147, Sanova) was added and diluted with water to 60 litres. This produced a concentration of approximately 1000 ppm at a pH between 2.39 and 2.67.

10.1.2.2 Peroxy Acetic Acids (PAA)

PAA used in poultry decontamination is a mixture of peroxyacetic acid (PAA), octanoic acid (OA), acetic acid (AA), hydrogen peroxide (HP), peroxyoctanoic acid (POA) and 1-hydroxyethylidene-1, 1-diphosphonic acid (HEDP) (SCVPH, 2003).

The product used for these trials (Proxitane 5, Solvay Interox) was supplied in the form of a bottle containing 5% of peracetic acid, 20% of HP and 10% of AA. The instruction from the supplier was to dissolve 1 litre of the product in 125 litre of water. These recommendations were followed, although it resulted in a slightly higher concentration than that recommended by EFSA. The solution we used contained 400 ppm of peracetic acid, 1600 ppm of HP and 800 ppm of AA.

10.1.2.3 Trisodium Phosphate (TSP)

For trials, TSP dodecahydrate powder (VWR International Ltd, UK) was diluted in water to a concentration of 12%, giving a highly alkaline solution of pH 12.4.

At the end of the trial, some of the TSP powder was found undissolved, resting at the bottom of the tank. Thus, a small investigation was carried out to assess the solubility of the TSP powder at different water temperatures. This study showed that the temperature of the water used to dissolve the TSP has an important influence on the solubility of the TSP. Moreover, it was noted that when the TSP was not agitated it had a tendency to agglomerate very quickly and that a very concentrated solution of TSP was particularly viscous.

Table 15. Study of the solubility of the TSP

	10°C	25°C	45°C
Quantity approximate of TSP dissolved in 200 ml of water in 1 hour (g)	24	50.7	126.6
Time approximate necessary to dissolve 12% of TSP in 200ml of water	More than 15 min *(Note: after 4 min the major part of the TSP is dissolved)*	2 min 45s	45s

10.1.2.4 Ozonated Water (OW)

The ozonation system used for the trials (IOCS05-C22, Pacific Ozone Technology, USA) is shown in **Error! Reference source not found.** The onboard ozone generator (SGC22) feeds compressed air into a pressure swing absorption oxygen separator unit, which in turn supplies oxygen to a corona discharge ozone module converting a percentage of that oxygen into ozone.

Figure 28. Ozone generator

The system used for these trials could produce in theory, 25 g of ozone per hour at a concentration of 3 to 7%. For the trials, the generator was set to generate the maximum ozone concentration that could be achieved with the generator. The measured concentrations were between 8.2 and 14.1 ppm. James (2003) states that decomposition of ozone is so rapid in the water phase of foods that its antimicrobial action is thought to take place mainly at the surface of treated products, however excessive ozone can cause rancidity.

10.1.3 Chicken carcasses

To maximise the probability of having Campylobacter-positive carcasses, trial carcasses were collected post-pluck from a poultry plant processing free-range chickens. Carcasses were packaged, five at a time, in large bags and transported directly to the experimental site (journey time c. 1 hour). Normally five chickens were used for each kind of treatment, five controls treated only with water to evaluate the washing effect and five controls without any treatment to see the overall effect of the treatments. The treatments of groups of five were replicated across different trial days to reduce effect of any flock/process/transport idiosyncrasies.

10.1.4 Sampling and enumeration of *Campylobacter* spp., Enterobacteriaceae and *Pseudomonas* spp.

The neck skin and the whole of the breast skin were removed from each carcass after treatment using sterile scissors or scalpel blades and forceps respectively, and each piece of skin put into a separate stomacher-80 bag, labelled and weighed. Fifty ml of Maximum Recovery Diluent (MRD) were added to the neck skins, and 30 ml of MRD added to each breast skin, and the samples were treated for 30 seconds with a Pulsifier (Microgen, Fleet Hampshire, UK). Decimal dilutions were prepared and surface plated onto selective agar media. See report sections 6.1.1 and 7.1.3 for methods used for *Campylobacter* spp. and Enterobacteriaceae. Enterobacteriaceae were enumerated in addition to Campylobacters as they are found on all carcasses, whether or not the birds had been colonised with Campylobacters, and they are also normally present in higher numbers. Presumptive *Pseudomonas* spp. were enumerated on CFC (cephaloridine fusidin cetrimide) agar (Oxoid CM 559 plus SR 103). Incubation was aerobic

at 25ºC for 48 hours. *Pseudomonas* spp. are important spoilage bacteria (numbers on carcasses at production are directly related to the length of aerobic shelf-life) and the effect of any carcass treatment on their numbers is therefore of particular interest to the poultry industry.

10.1.5 Statistical analysis

All bacterial counts were transformed to $\log_{10}$ cfu g^{-1} (of neck skin or breast skin) values for subsequent data analysis. Results were collated and analysed in MS Excel, using Student's T-test (2-tailed) at 99% confidence for all tests of significance.

10.2 Results

10.2.1 Overall Initial Anti-Microbial Efficacy Analysis

A total of 42 different treatments were applied (Table 16), with approximately 5 replicates and untreated controls for each trial condition on each trial date. The precise number of replicates analysed varied dependent on the amount of data rejected as suspect.

Table 16. Treatments and Identifiers

ID	Treatment	Configuration	Time in seconds (s) or minutes (m)	Dates
§01	ASC (Commercial)	Mist	10 s	2 Oct 07
§02	ASC (Commercial)	Mist	15 s	2, 8, 17 Oct 07, 16 Jan 08
§03	ASC (Homemade)	Mist	30 s	22, 24 Aug 07, 3, 5 Sep07
§04	ASC (Commercial)	Mist	30 s	19 Sep 07, 2 Oct 07, 16 Jan 08
§05	ASC (Commercial)	Mist+Wait+Rinse	15s+30s+30s	8 Oct 07
§06	ASC (Commercial)	Mist+Wait+Rinse	15s+5m+5s	17 Oct 07
§07	CD	Mist	30s	22 Aug 07, 12, 19 Sep 07
§08	CD	Wash	2.5s	19, 31 Jul 07
§09	CL	Wash	2.5s	19, 31 Jul 07
§10	CW	Mist	15s	8, 31 Oct 07, 27 Nov 07, 12 Dec 07, 16, 29 Jan 08
§11	CW	Mist	30s	22, 24 Aug 07, 3, 5, 12, 19 Sep 07, 2 Oct 07, 13, 21 Nov 07, 29 Jan 08, 20 May 08
§12	CW	Mist+Wait+Rinse	15s+5m+5s	17 Oct 07
§13	CW	OzoneNozzles	15s	5 Dec 07
§14	CW	OzoneNozzles	30s	5 Dec 07
§15	CW	OzoneNozzles	3m	13 Feb 08
§16	CW	Wash	2.5s	19, 31Jul 07
§17	EW	Mist	15s	21 Nov 07
§18	EW	Mist	30s	21 Nov 07
§19	HW	Wash	2.5s	19, 31 Jul 07
§20	Ozone	OzoneNozzles	15s	5 Dec 07
§21	Ozone	OzoneNozzles	30s	5 Dec 07
§22	Ozone	OzoneNozzles	3m	13 Feb 08
§23	PAA	Mist	15s	31 Oct 07, 27 Nov 07
§24	PAA	Mist	30s	31 Oct 07, 27 Nov 07
§25	PAA	Mist+Wait+Rinse	15s+5m+5s	31 Oct 07, 27 Nov 07
§26	(Stm)	Cool	15s	12 Mar 08, 9, 22 Apr08
§27	Stm	Stm	10s	12 Mar 08
§28	Stm	Stm	15s	12 Mar 08, 9, 22 Apr 08

§29	Stm	Stm+Cool	15s+15s	12 Mar 08, 9, 22 Apr 08
§30	Stm-Dry	Stm+Cool	15s+15s	9, 22 Apr 08
§31	TSP	Mist	15s	12 Dec 07, 29 Jan 08
§32	TSP	Mist	30s	13 Nov 07, 12 Dec 07, 29 Jan 08
§33	TSP	Mist+Rinse	15s+30s	12 Dec 07
§34	TSP	Mist+Rinse	30s+30s	12 Dec 07
§35	CW	Mist+Rinse	10s+5s	19 Aug 08, 9 Sep 08
§36	ASC (Commercial)	Mist+Rinse	10s+5s	19 Aug 08, 9 Sep 08
§37	ASC (Commercial)	Mist+Wait+Rinse	10s+5m+5s	19 Aug 08, 9 Sep 08
§38	ASC (Commercial)	Mist+Wait+Rinse	10s+30m+5s	19 Aug 08, 9 Sep 08
§39	ASC (Commercial)	Mist+Wait+Rinse	10s+60m+5s	19 Aug 08, 9 Sep 08
§40	CW	High Intensity Wash	5s	1 Oct 08
§41	CW	High Intensity Wash	15s	1 Oct 08
§42	CW	High Intensity Wash	30s	1 Oct 08

Key: ASC - Acidified Sodium Chlorite 1000 ppm; CD - Chlorine Dioxide; CL - Chlorine;
CW - Cold Water; EW- Electrolysed Water; HW - Hot Water; Ozone – Ozone; PAA - Peroxy Acetic Acids;
Stm – Steam; TSP- Trisodium phosphate.

The treatment mean and variance were calculated from results on all experimental days of each separate treatment. The mean and variance for the controls for each treatment were derived from the untreated controls for each of the days on which that particular treatment was performed. These collated results were then subdivided into six organism-group/skin-part combinations: (Campylobacters, Enterobacteriaceae, Pseudomonads) x (breastskin, neck skin). In addition to the $\log_{10}$ reduction, the proportion of samples where all plate counts were below the limit of detection was also calculated. These summary data are given in Table 17 to Table 22.

The three most effective chemical and three most effective physical treatments for each organism group/skin part combination, ranked by a). log reduction achieved by the treatment, b). proportion of samples after treatment below the limit of detection and c). levels remaining after treatment are given in Table 23.

Table 17. Summary of results for Campylobacters on breast skin

Campy Breast			Treated Log10				Control Log10					Redn	%<LoD				Significant Effect
Treatment	*Type*	*Repeat Days*	*n*	*#<LoD*	*mean*	*SD*	*n*	*#<LoD*	*mean*	*SD*			Treated	Control	Benefit		(P<0.01)
ID #1	Chem	1	5	5	< 0.43	0.26	5	0	2.00	0.87	>	1.57	100%	0%	**100%**	ID #1	y
ID #2	Chem	4	20	15	< 0.67	0.61	20	1	< 1.52	0.67	~	0.85	75%	5%	**70%**	ID #2	y
ID #3	Chem	4	35	3	< 1.58	0.67	20	0	2.39	0.76	>	0.81	9%	0%	**9%**	ID #3	y
ID #4	Chem	3	15	14	< 0.45	0.17	15	0	1.73	0.62	>	1.28	93%	0%	**93%**	ID #4	y
ID #5	Chem	1	5	1	< 1.34	0.66	5	1	< 0.97	0.34	~	-0.37	20%	20%	0%	ID #5	n
ID #6	Chem	1	5	4	< 0.72	0.68	5	0	1.24	0.55	>	0.52	80%	0%	**80%**	ID #6	n
ID #7	Chem	3	20	2	< 1.46	0.53	15	0	1.59	0.52	>	0.13	10%	0%	**10%**	ID #7	n
ID #8	Chem	1	5	0	1.93	0.48	10	0	1.85	0.43		-0.08	0%	0%	0%	ID #8	n
ID #9	Chem	1	5	0	2.05	0.84	10	0	1.85	0.43		-0.20	0%	0%	0%	ID #9	n
ID #10	Phys	6	30	1	< 2.25	0.73	30	1	< 2.28	0.90	~	0.03	3%	3%	0%	ID #10	n
ID #11	Phys	11	55	0	1.93	0.52	55	1	< 2.12	0.79	<	0.19	0%	2%	-2%	ID #11	n
ID #12	Phys	1	5	0	1.60	0.42	5	0	1.24	0.55		-0.36	0%	0%	0%	ID #12	n
ID #13	Phys	1	5	0	1.60	0.33	5	0	2.28	0.14		0.68	0%	0%	0%	ID #13	y
ID #14	Phys	1	5	0	2.02	0.33	5	0	2.28	0.14		0.26	0%	0%	0%	ID #14	n
ID #15	Phys	1	5	0	2.02	0.13	5	0	1.72	0.51		-0.30	0%	0%	0%	ID #15	n
ID #16	Phys	1	5	0	2.07	0.15	10	0	1.85	0.43		-0.22	0%	0%	0%	ID #16	n
ID #17	Chem	1	5	0	2.04	0.40	5	0	1.48	0.69		-0.56	0%	0%	0%	ID #17	n
ID #18	Chem	1	5	0	1.67	0.56	5	0	1.48	0.69		-0.19	0%	0%	0%	ID #18	n
ID #19	Phys	1	5	0	1.78	0.66	10	0	1.85	0.43		0.07	0%	0%	0%	ID #19	n
ID #20	Chem	1	5	0	1.71	0.28	5	0	2.28	0.14		0.57	0%	0%	0%	ID #20	y
ID #21	Chem	1	5	1	< 1.43	0.79	5	0	2.28	0.14	>	0.85	20%	0%	**20%**	ID #21	n
ID #22	Chem	1	5	0	1.80	0.74	5	0	1.72	0.51		-0.08	0%	0%	0%	ID #22	n
ID #23	Chem	2	10	0	2.12	0.83	10	0	2.93	0.94		0.81	0%	0%	0%	ID #23	n
ID #24	Chem	2	10	2	< 1.78	0.75	10	0	2.93	0.94	>	1.15	20%	0%	**20%**	ID #24	n
ID #25	Chem	2	10	0	2.11	0.45	10	0	2.93	0.94		0.82	0%	0%	0%	ID #25	n
ID #26	Phys	3	14	5	< 1.49	0.95	15	1	< 2.15	0.83	~	0.66	36%	7%	**29%**	ID #26	n
ID #27	Phys	1	5	0	1.50	0.45	5	0	1.31	0.42		-0.19	0%	0%	0%	ID #27	n
ID #28	Phys	3	15	8	< 0.87	0.63	15	1	< 2.15	0.83	~	1.28	53%	7%	**47%**	ID #28	y
ID #29	Phys	3	15	9	< 0.91	0.55	15	1	< 2.15	0.83	~	1.24	60%	7%	**53%**	ID #29	y
ID #30	Phys	2	10	2	< 1.32	1.04	10	0	2.13	0.45	>	0.81	20%	0%	**20%**	ID #30	n
ID #31	Chem	2	10	0	1.92	0.61	10	0	2.50	0.21		0.58	0%	0%	0%	ID #31	n
ID #32	Chem	3	15	10	< 0.79	0.38	15	1	< 2.16	0.57	~	1.37	67%	7%	**60%**	ID #32	y
ID #33	Chem	1	5	0	1.79	0.35	5	0	2.37	0.16		0.58	0%	0%	0%	ID #33	n
ID #34	Chem	1	5	0	1.50	0.28	5	0	2.37	0.16		0.87	0%	0%	0%	ID #34	y
ID #35	Phys	2	10	0	2.54	0.27	10	0	2.65	0.36		0.11	0%	0%	0%	ID #35	n
ID #36	Chem	2	9	2	< 1.39	0.79	10	0	2.65	0.36	>	1.26	22%	0%	**22%**	ID #36	y
ID #37	Chem	2	10	4	< 0.83	0.62	10	0	2.65	0.36	>	1.82	40%	0%	**40%**	ID #37	y
ID #38	Chem	2	10	1	< 1.38	0.65	10	0	2.65	0.36	>	1.27	10%	0%	**10%**	ID #38	y
ID #39	Chem	2	10	0	1.70	0.63	10	0	2.65	0.36		0.95	0%	0%	0%	ID #39	y
ID #40	Phys	1	5	0	2.13	0.48	5	0	2.71	0.39		0.58	0%	0%	0%	ID #40	n
ID #41	Phys	1	5	0	1.98	1.09	5	0	2.71	0.39		0.73	0%	0%	0%	ID #41	n
ID #42	Phys	1	5	0	2.12	0.15	5	0	2.71	0.39		0.59	0%	0%	0%	ID #42	n

Table 18. Summary of results for Campylobacters on neck skin

Campy Neck			Treated Log10				Control Log10					Redn	%<LoD				Significant Effect
Treatment	*Type*	*Repeat Days*	*n*	*#<LoD*	*mean*	*SD*	*n*	*#<LoD*	*mean*	*SD*			Treated	Control	Benefit		(P<0.01)
ID#01	Chem	1	5	1	< 1.41	1.47	5	0	2.86	1.37	>	1.45	20%	0%	**20%**	ID#01	n
ID#02	Chem	4	20	13	< 0.96	0.92	20	0	2.41	0.87	>	1.45	65%	0%	**65%**	ID#02	y
ID#03	Chem	4	35	2	< 1.86	0.69	20	0	2.90	0.92	>	1.04	6%	0%	**6%**	ID#03	y
ID#04	Chem	3	15	7	< 0.85	0.60	15	0	2.45	0.91	>	1.60	47%	0%	**47%**	ID#04	y
ID#05	Chem	1	5	1	< 1.48	0.89	5	0	1.87	0.49	>	0.39	20%	0%	**20%**	ID#05	n
ID#06	Chem	1	5	1	< 1.55	0.89	5	0	2.40	0.74	>	0.85	20%	0%	**20%**	ID#06	n
ID#07	Chem	3	20	0	2.11	0.44	15	0	1.96	0.41		-0.15	0%	0%	0%	ID#07	n
ID#08	Chem	2	10	0	2.52	0.83	20	0	2.46	0.67		-0.06	0%	0%	0%	ID#08	n
ID#09	Chem	2	10	0	2.63	0.91	20	0	2.46	0.67		-0.17	0%	0%	0%	ID#09	n
ID#10	Phys	6	30	0	2.78	0.84	30	0	3.01	0.85		0.38	0%	0%	0%	ID#10	n
ID#11	Phys	11	55	0	2.41	0.61	55	0	2.81	0.85		0.03	0%	0%	0%	ID#11	Y
ID#12	Phys	1	5	1	< 2.21	1.17	5	0	2.40	0.74	>	0.19	20%	0%	**20%**	ID#12	n
ID#13	Phys	1	5	0	2.43	0.04	5	0	2.98	0.46		0.55	0%	0%	0%	ID#13	n
ID#14	Phys	1	5	0	2.91	0.60	5	0	2.98	0.46		0.07	0%	0%	0%	ID#14	n
ID#15	Phys	1	5	0	3.02	0.45	5	0	1.98	0.14		-1.04	0%	0%	0%	ID#15	y
ID#16	Phys	2	10	0	2.72	0.78	20	0	2.46	0.67		-0.26	0%	0%	0%	ID#16	n
ID#17	Chem	1	5	0	2.26	0.60	5	0	2.57	0.30		0.31	0%	0%	0%	ID#17	n
ID#18	Chem	1	5	0	2.23	0.48	5	0	2.57	0.30		0.34	0%	0%	0%	ID#18	n
ID#19	Phys	2	10	0	2.81	1.18	20	0	2.46	0.67		-0.35	0%	0%	0%	ID#19	n
ID#20	Chem	1	5	0	2.48	0.67	5	0	2.98	0.46		0.50	0%	0%	0%	ID#20	n
ID#21	Chem	1	5	0	2.77	0.46	5	0	2.98	0.46		0.21	0%	0%	0%	ID#21	n
ID#22	Chem	1	5	0	2.71	0.86	5	0	1.98	0.14		-0.73	0%	0%	0%	ID#22	n
ID#23	Chem	2	10	0	2.62	0.73	10	0	3.58	0.84		0.96	0%	0%	0%	ID#23	n
ID#24	Chem	2	10	0	2.61	0.89	10	0	3.58	0.84		0.97	0%	0%	0%	ID#24	n
ID#25	Chem	2	10	0	2.47	0.35	10	0	3.58	0.84		1.11	0%	0%	0%	ID#25	y
ID#26	Phys	3	14	0	2.49	1.01	15	0	2.58	1.03		0.09	0%	0%	0%	ID#26	n
ID#27	Phys	1	5	0	2.35	0.22	5	0	1.57	0.40		-0.78	0%	0%	0%	ID#27	y
ID#28	Phys	3	15	0	2.05	0.85	15	0	2.58	1.03		0.53	0%	0%	0%	ID#28	n
ID#29	Phys	3	15	1	< 2.09	1.03	15	0	2.58	1.03	>	0.49	7%	0%	**7%**	ID#29	n
ID#30	Phys	2	10	0	2.64	0.97	10	0	3.19	0.80		0.55	0%	0%	0%	ID#30	n
ID#31	Chem	2	10	2	< 1.89	1.22	10	0	3.25	0.28	>	1.36	20%	0%	**20%**	ID#31	Y
ID#32	Chem	3	15	11	< 0.81	0.74	15	0	3.22	0.46	>	2.41	73%	0%	**73%**	ID#32	y
ID#33	Chem	1	5	0	2.25	0.42	5	0	3.24	0.18		0.99	0%	0%	0%	ID#33	y
ID#34	Chem	1	5	0	2.11	0.52	5	0	3.24	0.18		1.13	0%	0%	0%	ID#34	y
ID#35	Phys	2	10	0	2.88	0.32	10	0	2.88	0.44		0.00	0%	0%	0%	ID#35	-
ID#36	Chem	2	9	1	< 1.80	0.73	10	0	2.88	0.44	>	1.08	11%	0%	**11%**	ID#36	y
ID#37	Chem	2	10	1	< 1.76	0.61	10	0	2.88	0.44	>	1.12	10%	0%	**10%**	ID#37	y
ID#38	Chem	2	10	0	2.33	0.54	10	0	2.88	0.44		0.55	0%	0%	0%	ID#38	n
ID#39	Chem	2	10	2	< 1.89	1.07	10	0	2.88	0.44	>	0.99	20%	0%	**20%**	ID#39	n
ID#40	Phys	1	5	0	2.91	0.49	5	0	3.05	0.40		0.14	0%	0%	0%	ID#40	n
ID#41	Phys	1	5	0	2.68	0.40	5	0	3.05	0.40		0.37	0%	0%	0%	ID#41	n
ID#42	Phys	1	5	0	2.93	0.49	5	0	3.05	0.40		0.12	0%	0%	0%	ID#42	n

Table 19. Summary of results for Enterobacteriaceae on breast skin

Entero Breast			Treated Log10				Control Log10					%<LoD				Significant Effect
Treatment	*Type*	*Repeat Days*	*n*	*#<LoD*	*mean*	*SD*	*n*	*#<LoD*	*mean*	*SD*	Redn	Treated	Control	Benefit		(P<0.01)
ID#01	Chem	1	5	2	< 0.61	0.32	5	0	3.01	0.76	> 2.40	40%	0%	**40%**	ID#01	y
ID#02	Chem	4	20	9	< 1.31	0.87	20	0	2.89	0.54	> 1.58	45%	0%	**45%**	ID#02	y
ID#03	Chem	4	35	0	4.12	1.18	20	0	4.44	1.12	0.32	0%	0%	0%	ID#03	n
ID#04	Chem	3	15	7	< 1.17	0.87	15	0	3.27	0.52	> 2.10	47%	0%	**47%**	ID#04	y
ID#05	Chem	1	5	0	2.70	0.34	5	0	2.39	0.19	-0.31	0%	0%	0%	ID#05	n
ID#06	Chem	1	5	0	1.99	0.39	5	0	2.79	0.30	0.80	0%	0%	0%	ID#06	N
ID#07	Chem	3	20	0	3.42	0.45	15	0	3.91	0.75	0.49	0%	0%	0%	ID#07	n
ID#08	Chem	1	5	0	2.09	0.67	10	0	2.03	0.22	-0.06	0%	0%	0%	ID#08	n
ID#09	Chem	1	5	0	2.27	0.55	10	0	2.03	0.22	-0.24	0%	0%	0%	ID#09	n
ID#10	Phys	6	30	0	3.53	0.73	30	0	3.30	0.75	1.03	0%	0%	0%	ID#10	n
ID#11	Phys	11	55	0	3.66	0.88	55	0	3.79	1.06	0.26	0%	0%	0%	ID#11	n
ID#12	Phys	1	5	0	3.18	0.39	5	0	2.79	0.30	-0.39	0%	0%	0%	ID#12	n
ID#13	Phys	1	5	0	3.33	0.21	5	0	2.86	0.46	-0.47	0%	0%	0%	ID#13	n
ID#14	Phys	1	5	0	3.02	0.37	5	0	2.86	0.46	-0.16	0%	0%	0%	ID#14	n
ID#15	Phys	1	5	0	2.66	0.52	5	0	3.06	0.60	0.40	0%	0%	0%	ID#15	n
ID#16	Phys	1	5	0	2.16	0.19	10	0	2.03	0.22	-0.13	0%	0%	0%	ID#16	n
ID#17	Chem	1	5	0	3.75	0.29	5	0	4.06	0.52	0.31	0%	0%	0%	ID#17	n
ID#18	Chem	1	5	0	4.09	0.10	5	0	4.06	0.52	-0.03	0%	0%	0%	ID#18	n
ID#19	Phys	1	5	1	< 1.88	0.69	10	0	2.03	0.22	> 0.15	20%	0%	**20%**	ID#19	n
ID#20	Chem	1	5	0	3.17	0.24	5	0	2.86	0.46	-0.31	0%	0%	0%	ID#20	n
ID#21	Chem	1	5	0	3.26	0.36	5	0	2.86	0.46	-0.40	0%	0%	0%	ID#21	n
ID#22	Chem	1	5	0	2.87	0.25	5	0	3.60	0.60	0.73	0%	0%	0%	ID#22	n
ID#23	Chem	2	10	0	3.17	0.80	10	0	3.36	0.83	0.19	0%	0%	0%	ID#23	n
ID#24	Chem	2	10	0	2.76	0.53	10	0	3.36	0.83	0.60	0%	0%	0%	ID#24	n
ID#25	Chem	2	10	0	3.62	0.50	10	0	3.36	0.83	-0.26	0%	0%	0%	ID#25	n
ID#26	Phys	3	14	3	< 2.53	0.27	15	0	3.28	0.36	> 0.75	21%	0%	**21%**	ID#26	y
ID#27	Phys	1	5	0	2.88	0.31	5	0	3.18	0.39	0.30	0%	0%	0%	ID#27	n
ID#28	Phys	3	15	1	< 2.26	0.99	15	0	3.28	0.36	> 1.02	7%	0%	**7%**	ID#28	y
ID#29	Phys	3	15	2	< 1.87	0.88	15	0	3.28	0.36	> 1.41	13%	0%	**13%**	ID#29	y
ID#30	Phys	2	10	0	2.12	0.91	10	0	3.10	0.28	0.98	0%	0%	0%	ID#30	Y
ID#31	Chem	2	10	0	3.46	0.89	10	0	3.67	0.68	0.21	0%	0%	0%	ID#31	n
ID#32	Chem	3	15	4	< 2.13	1.26	15	0	3.14	0.99	> 1.01	27%	0%	**27%**	ID#32	n
ID#33	Chem	1	5	0	2.73	0.27	5	0	3.07	0.19	0.34	0%	0%	0%	ID#33	n
ID#34	Chem	1	5	0	2.80	0.27	5	0	3.07	0.19	0.27	0%	0%	0%	ID#34	n
ID#35	Phys	2	10	0	3.98	0.78	10	0	3.70	0.53	-0.28	0%	0%	0%	ID#35	n
ID#36	Chem	2	9	0	3.14	0.62	10	0	3.70	0.53	0.56	0%	0%	0%	ID#36	n
ID#37	Chem	2	10	0	3.53	0.36	10	0	3.70	0.53	0.17	0%	0%	0%	ID#37	n
ID#38	Chem	2	10	0	2.92	0.69	10	0	3.70	0.53	0.78	0%	0%	0%	ID#38	N
ID#39	Chem	2	10	0	3.11	0.59	10	0	3.70	0.53	0.59	0%	0%	0%	ID#39	n
ID#40	Phys	1	5	0	3.19	0.17	5	0	3.26	0.19	0.07	0%	0%	0%	ID#40	n
ID#41	Phys	1	5	0	3.43	0.49	5	0	3.27	0.19	-0.16	0%	0%	0%	ID#41	n
ID#42	Phys	1	5	0	3.46	0.25	5	0	3.26	0.19	-0.20	0%	0%	0%	ID#42	n

Table 20. Summary of results for Enterobacteriaceae on neck skin

Entero Neck			Treated Log10					Control Log10					Redn	Treated %<LoD				Significant Effect
Treatment	*Type*	*Repeat Days*	*n*	*#<LoD*		*mean*	*SD*	*n*	*#<LoD*		*mean*	*SD*		Treated	Control	Benefit		(P<0.01)
ID#01	Chem	1	5	1	<	1.41	0.87	5	0		3.59	0.83	> 2.18	20%	0%	**20%**	ID#01	y
ID#02	Chem	4	20	3	<	1.81	1.00	20	0		3.58	0.67	> 1.77	15%	0%	**15%**	ID#02	y
ID#03	Chem	4	35	0		3.84	1.27	20	0		4.54	1.13	0.70	0%	0%	0%	ID#03	n
ID#04	Chem	3	15	1	<	2.07	0.80	15	0		3.96	0.64	> 1.89	7%	0%	**7%**	ID#04	y
ID#05	Chem	1	5	0		2.23	0.55	5	0		3.14	0.25	0.91	0%	0%	0%	ID#05	n
ID#06	Chem	1	5	0		2.78	0.32	5	0		3.20	0.17	0.42	0%	0%	0%	ID#06	n
ID#07	Chem	3	20	0		3.59	0.52	15	0		4.10	0.84	0.51	0%	0%	0%	ID#07	n
ID#08	Chem	2	10	0		2.70	0.38	20	0		2.84	0.58	0.14	0%	0%	0%	ID#08	n
ID#09	Chem	2	10	0		3.11	0.46	20	0		2.84	0.58	-0.27	0%	0%	0%	ID#09	n
ID#10	Phys	6	30	0		3.94	0.58	30	0		4.01	0.83	0.07	0%	0%	0%	ID#10	n
ID#11	Phys	11	55	0		3.78	0.79	55	0		4.16	0.94	0.38	0%	0%	0%	ID#11	n
ID#12	Phys	1	5	0		3.11	0.51	5	0		3.20	0.17	0.09	0%	0%	0%	ID#12	n
ID#13	Phys	1	5	0		3.69	0.32	5	0		3.55	0.33	-0.14	0%	0%	0%	ID#13	n
ID#14	Phys	1	5	0		3.72	0.40	5	0		3.55	0.33	-0.17	0%	0%	0%	ID#14	n
ID#15	Phys	1	5	0		3.41	0.31	5	0		3.36	0.34	-0.05	0%	0%	0%	ID#15	n
ID#16	Phys	2	10	0		2.91	0.44	20	0		2.84	0.58	-0.07	0%	0%	0%	ID#16	n
ID#17	Chem	1	5	0		3.86	0.51	5	0		3.48	0.35	-0.38	0%	0%	0%	ID#17	n
ID#18	Chem	1	5	0		4.05	0.39	5	0		3.48	0.35	-0.57	0%	0%	0%	ID#18	n
ID#19	Phys	2	10	0		3.21	0.36	20	0		2.84	0.58	-0.37	0%	0%	0%	ID#19	n
ID#20	Chem	1	5	0		3.68	0.50	5	0		3.55	0.33	-0.13	0%	0%	0%	ID#20	n
ID#21	Chem	1	5	0		3.86	0.58	5	0		3.55	0.33	-0.31	0%	0%	0%	ID#21	n
ID#22	Chem	1	5	0		3.72	0.51	5	0		3.36	0.34	-0.36	0%	0%	0%	ID#22	n
ID#23	Chem	2	10	0		3.17	0.64	10	0		3.97	0.69	0.80	0%	0%	0%	ID#23	n
ID#24	Chem	2	10	0		3.21	0.46	10	0		3.97	0.69	0.76	0%	0%	0%	ID#24	n
ID#25	Chem	2	10	0		3.75	0.48	10	0		3.97	0.69	0.22	0%	0%	0%	ID#25	n
ID#26	Phys	3	14	0		3.78	0.61	15	0		3.72	0.48	-0.06	0%	0%	0%	ID#26	n
ID#27	Phys	1	5	0		3.71	0.74	5	0		3.70	0.43	-0.01	0%	0%	0%	ID#27	n
ID#28	Phys	3	15	0		3.70	0.57	15	0		3.72	0.48	0.02	0%	0%	0%	ID#28	n
ID#29	Phys	3	15	0		3.61	0.63	15	0		3.72	0.48	0.11	0%	0%	0%	ID#29	n
ID#30	Phys	2	10	0		3.65	0.79	10	0		3.88	0.46	0.23	0%	0%	0%	ID#30	n
ID#31	Chem	2	10	0		2.59	1.62	10	0		4.29	1.04	1.70	0%	0%	0%	ID#31	n
ID#32	Chem	3	15	10	<	0.88	0.83	15	0		4.17	0.90	> 3.29	67%	0%	**67%**	ID#32	y
ID#33	Chem	1	5	0		2.86	0.48	5	0		3.34	0.21	0.48	0%	0%	0%	ID#33	n
ID#34	Chem	1	5	0		2.89	0.22	5	0		3.34	0.21	0.45	0%	0%	0%	ID#34	n
ID#35	Phys	2	10	0		4.32	0.62	10	0		4.01	0.57	-0.31	0%	0%	0%	ID#35	n
ID#36	Chem	2	9	0		3.30	0.45	10	0		4.01	0.57	0.71	0%	0%	0%	ID#36	n
ID#37	Chem	2	10	0		3.80	0.43	10	0		4.01	0.57	0.21	0%	0%	0%	ID#37	n
ID#38	Chem	2	10	0		3.37	0.69	10	0		4.01	0.57	0.64	0%	0%	0%	ID#38	n
ID#39	Chem	2	10	0		3.32	0.76	10	0		4.01	0.57	0.69	0%	0%	0%	ID#39	n
ID#40	Phys	1	5	0		3.79	0.60	5	0		3.65	0.37	-0.14	0%	0%	0%	ID#40	n
ID#41	Phys	1	5	0		3.64	0.46	5	0		3.65	0.37	0.01	0%	0%	0%	ID#41	n
ID#42	Phys	1	5	0		3.85	0.24	5	0		3.65	0.37	-0.20	0%	0%	0%	ID#42	n

Table 21. Summary of results for Pseudomonads on breast skin

Pseudo Breast			Treated Log10				Control Log10					Redn	%<LoD				Significant Effect
Treatment	*Type*	*Repeat Days*	*n*	*#<LoD*	*mean*	*SD*	*n*	*#<LoD*	*mean*	*SD*			Treated	Control	Benefit		(P<0.01)
ID#01	Chem	1	5	2	< 1.29	0.78	5	0	3.29	0.12	>	2.00	40%	0%	**40%**	ID#01	y
ID#02	Chem	4	20	5	< 1.33	0.70	20	0	2.49	0.65	>	1.16	25%	0%	**25%**	ID#02	y
ID#03	Chem	4	35	0	3.15	1.03	20	0	3.35	0.90		0.20	0%	0%	0%	ID#03	n
ID#04	Chem	3	15	3	< 1.57	0.71	15	0	2.78	0.48	>	1.21	20%	0%	**20%**	ID#04	y
ID#05	Chem	1	5	0	1.56	0.40	5	0	1.69	0.41		0.13	0%	0%	0%	ID#05	n
ID#06	Chem	1	5	1	< 1.59	0.81	5	0	2.55	0.38	>	0.96	20%	0%	**20%**	ID#06	n
ID#07	Chem	3	20	0	2.44	0.46	15	0	2.82	0.55		0.38	0%	0%	0%	ID#07	n
ID#08	Chem	1	5	0	1.94	0.36	10	0	2.60	0.50		0.66	0%	0%	0%	ID#08	n
ID#09	Chem	1	5	0	1.94	0.36	10	0	2.60	0.50		0.66	0%	0%	0%	ID#09	n
ID#10	Phys	6	30	0	2.42	0.64	30	0	2.29	0.63		-0.13	0%	0%	0%	ID#10	n
ID#11	Phys	11	55	0	3.07	0.95	55	2	< 2.78	0.90	<	-0.29	0%	4%	-4%	ID#11	n
ID#12	Phys	1	5	0	2.55	0.06	5	0	2.55	0.38		0.00	0%	0%	0%	ID#12	n
ID#13	Phys	1	5	0	2.38	0.27	5	0	2.05	0.42		-0.33	0%	0%	0%	ID#13	n
ID#14	Phys	1	5	0	2.11	0.26	5	0	2.05	0.42		-0.06	0%	0%	0%	ID#14	n
ID#15	Phys	1	5	0	1.45	0.30	5	0	2.99	0.51		1.54	0%	0%	0%	ID#15	y
ID#16	Phys	1	5	0	3.91	0.48	10	0	2.60	0.50		-1.31	0%	0%	0%	ID#16	y
ID#17	Chem	1	5	0	2.68	0.29	5	0	2.36	0.19		-0.32	0%	0%	0%	ID#17	n
ID#18	Chem	1	5	0	2.53	0.41	5	0	2.36	0.19		-0.17	0%	0%	0%	ID#18	n
ID#19	Phys	1	5	0	1.80	0.39	10	0	2.60	0.50		0.80	0%	0%	0%	ID#19	y
ID#20	Chem	1	5	0	2.08	0.16	5	0	2.05	0.42		-0.03	0%	0%	0%	ID#20	n
ID#21	Chem	1	5	0	2.21	0.20	5	0	2.05	0.42		-0.16	0%	0%	0%	ID#21	n
ID#22	Chem	1	5	0	2.34	0.62	5	0	2.99	0.51		0.65	0%	0%	0%	ID#22	n
ID#23	Chem	2	10	0	2.00	0.52	10	0	2.37	0.67		0.37	0%	0%	0%	ID#23	n
ID#24	Chem	2	10	0	1.81	0.52	10	0	2.37	0.67		0.56	0%	0%	0%	ID#24	n
ID#25	Chem	2	10	0	2.55	0.28	10	0	2.37	0.67		-0.18	0%	0%	0%	ID#25	n
ID#26	Phys	3	15	5	< 2.58	1.56	15	0	2.46	0.44	~	-0.12	33%	0%	**33%**	ID#26	n
ID#27	Phys	1	5	0	1.33	0.34	5	0	2.39	0.36		1.06	0%	0%	0%	ID#27	y
ID#28	Phys	3	15	6	< 1.14	0.77	15	0	2.46	0.44	>	1.32	40%	0%	**40%**	ID#28	y
ID#29	Phys	3	15	3	< 2.20	1.11	15	0	2.46	0.44	>	0.26	20%	0%	**20%**	ID#29	n
ID#30	Phys	2	10	0	2.68	0.60	10	0	2.27	0.45		-0.41	0%	0%	0%	ID#30	n
ID#31	Chem	2	10	1	< 2.02	0.90	10	0	2.45	0.68	>	0.43	10%	0%	**10%**	ID#31	n
ID#32	Chem	3	15	6	< 1.23	0.76	15	2	< 1.98	0.90	~	0.75	40%	13%	**27%**	ID#32	n
ID#33	Chem	1	5	0	1.42	0.42	5	0	1.83	0.23		0.41	0%	0%	0%	ID#33	n
ID#34	Chem	1	5	0	1.37	0.61	5	0	1.83	0.23		0.46	0%	0%	0%	ID#34	n
ID#35	Phys	2	10	0	3.24	0.44	10	0	2.99	0.58		-0.25	0%	0%	0%	ID#35	n
ID#36	Chem	2	9	0	2.70	0.46	10	0	2.99	0.58		0.29	0%	0%	0%	ID#36	n
ID#37	Chem	2	10	0	2.33	0.55	10	0	2.99	0.58		0.66	0%	0%	0%	ID#37	n
ID#38	Chem	2	10	0	2.00	0.70	10	0	2.99	0.58		0.99	0%	0%	0%	ID#38	y
ID#39	Chem	2	10	0	2.08	0.75	10	0	2.99	0.58		0.91	0%	0%	0%	ID#39	y
ID#40	Phys	1	5	0	2.35	0.39	5	0	2.21	0.12		-0.14	0%	0%	0%	ID#40	n
ID#41	Phys	1	5	0	1.90	0.46	5	0	2.21	0.12		0.31	0%	0%	0%	ID#41	n
ID#42	Phys	1	5	0	2.22	0.32	5	0	2.21	0.12		-0.01	0%	0%	0%	ID#42	n

Table 22. Summary of results for Pseudomonads on neck skin

Pseudo Neck			Treated Log10				Control Log10					Redn	%<LoD				Confidence
																	>99%
Treatment	*Type*	*Repeat Days*	*n*	*#<LoD*	*mean*	*SD*	*n*	*#<LoD*	*mean*	*SD*			Treated	Control	Benefit		
ID #1	Chem	1	5	0	2.24	0.13	5	0	3.37	0.24		1.13	0%	0%	0%	ID #1	y
ID #2	Chem	4	20	0	2.20	0.51	20	0	2.94	0.47		0.74	0%	0%	0%	ID #2	y
ID #3	Chem	4	35	0	3.12	1.12	20	0	3.73	0.95		0.61	0%	0%	0%	ID #3	n
ID #4	Chem	3	15	0	2.33	0.60	15	0	3.23	0.37		0.90	0%	0%	0%	ID #4	y
ID #5	Chem	1	5	0	2.16	0.24	5	0	2.43	0.23		0.27	0%	0%	0%	ID #5	n
ID #6	Chem	1	5	0	2.76	0.30	5	0	2.78	0.18		0.02	0%	0%	0%	ID #6	n
ID #7	Chem	3	20	0	2.88	0.30	15	0	2.94	0.50		0.06	0%	0%	0%	ID #7	n
ID #8	Chem	2	10	0	2.76	0.55	20	0	3.05	0.50		0.29	0%	0%	0%	ID #8	n
ID #9	Chem	2	10	0	3.13	0.52	20	0	3.05	0.50		-0.08	0%	0%	0%	ID #9	n
ID #10	Phys	6	30	0	2.92	0.54	30	0	2.91	0.56		-0.01	0%	0%	0%	ID #10	n
ID #11	Phys	11	55	0	3.35	0.79	55	0	3.22	0.77		-0.13	0%	0%	0%	ID #11	n
ID #12	Phys	1	5	0	2.87	0.35	5	0	2.78	0.18		-0.09	0%	0%	0%	ID #12	n
ID #13	Phys	1	5	0	2.73	0.24	5	0	2.76	0.28		0.03	0%	0%	0%	ID #13	n
ID #14	Phys	1	5	0	2.79	0.27	5	0	2.76	0.28		-0.03	0%	0%	0%	ID #14	n
ID #15	Phys	1	5	0	2.25	0.36	5	0	3.38	0.08		1.13	0%	0%	0%	ID #15	y
ID #16	Phys	2	10	0	4.09	0.24	20	0	3.05	0.50		-1.04	0%	0%	0%	ID #16	y
ID #17	Chem	1	5	0	2.87	0.18	5	0	2.86	0.11		-0.01	0%	0%	0%	ID #17	n
ID #18	Chem	1	5	0	2.90	0.23	5	0	2.86	0.11		-0.04	0%	0%	0%	ID #18	n
ID #19	Phys	2	10	0	3.22	0.59	20	0	3.05	0.50		-0.17	0%	0%	0%	ID #19	n
ID #20	Chem	1	5	0	2.81	0.44	5	0	2.76	0.28		-0.05	0%	0%	0%	ID #20	n
ID #21	Chem	1	5	0	2.59	0.29	5	0	2.76	0.28		0.17	0%	0%	0%	ID #21	n
ID #22	Chem	1	5	0	2.65	0.30	5	0	3.38	0.08		0.73	0%	0%	0%	ID #22	y
ID #23	Chem	2	10	0	2.29	0.30	10	0	3.08	0.53		0.79	0%	0%	0%	ID #23	y
ID #24	Chem	2	10	0	2.33	0.22	10	0	3.08	0.53		0.75	0%	0%	0%	ID #24	y
ID #25	Chem	2	10	0	2.94	0.45	10	0	3.08	0.53		0.14	0%	0%	0%	ID #25	n
ID #26	Phys	3	14	0	2.98	1.25	15	0	2.66	0.53		-0.32	0%	0%	0%	ID #26	n
ID #27	Phys	1	5	0	2.33	0.48	5	0	2.70	0.43		0.37	0%	0%	0%	ID #27	n
ID #28	Phys	3	15	0	2.24	0.83	15	0	2.66	0.53		0.42	0%	0%	0%	ID #28	n
ID #29	Phys	3	15	0	3.27	0.88	15	0	2.66	0.53		-0.61	0%	0%	0%	ID #29	n
ID #30	Phys	2	10	0	3.31	0.61	10	0	2.97	0.47		-0.34	0%	0%	0%	ID #30	n
ID #31	Chem	2	10	1	< 1.92	1.06	10	0	2.85	0.62	>	0.93	10%	0%	**10%**	ID #31	n
ID #32	Chem	3	15	8	< 0.81	0.56	15	0	2.82	0.51	>	2.01	53%	0%	**53%**	ID #32	y
ID #33	Chem	1	5	0	1.59	0.34	5	0	2.35	0.24		0.76	0%	0%	0%	ID #33	y
ID #34	Chem	1	5	0	1.53	0.56	5	0	2.35	0.24		0.82	0%	0%	0%	ID #34	n
ID #35	Phys	2	10	0	3.46	0.70	10	0	3.42	0.69		-0.04	0%	0%	0%	ID #35	n
ID #36	Chem	2	9	0	2.78	0.41	10	0	3.42	0.69		0.64	0%	0%	0%	ID #36	n
ID #37	Chem	2	10	0	2.43	0.38	10	0	3.42	0.69		0.99	0%	0%	0%	ID #37	y
ID #38	Chem	2	10	1	< 2.30	0.80	10	0	3.42	0.69	>	1.12	10%	0%	**10%**	ID #38	y
ID #39	Chem	2	10	0	2.38	0.51	10	0	3.42	0.69		1.04	0%	0%	0%	ID #39	y
ID #40	Phys	1	5	0	2.97	0.90	5	0	2.39	0.24		-0.58	0%	0%	0%	ID #40	n
ID #41	Phys	1	5	0	2.58	0.42	5	0	2.39	0.24		-0.19	0%	0%	0%	ID #41	n
ID #42	Phys	1	5	0	2.50	0.37	5	0	2.39	0.24		-0.11	0%	0%	0%	ID #42	n

Table 23. Most effective antimicrobial treatments (see Table 16 for abbreviations. Numbers in red refer to tests listed in Tables 17 - 22)

Campylobacters on breast skin	**Rated by proportion of samples <LoD**	**Rated by Reduction**	**Rated by Final Levels Remaining**
Physical Treatments	**#29** Stm+Cool 15s+15s (60%, n=15) **#28** Stm 15s (53%, n=15) **#26** (no Stm+) Cool Only (0s+)15s (36%, n=15)	**#28** Stm 15s (~1.28 log reduction at >99% confidence, n=15) **#29** Stm+Cool 15s+15s (~1.24 log reduction at >99% confidence, n=15) **#30** Dry Stm+Cool 15s+15s (>0.81 log reduction at <99% confidence, n=10)	**#28** Stm 15s (<0.87 log remaining, n=15) **#29** Stm+Cool 15s+15s (<0.91 log remaining, n=15) **#30** Dry Stm+Cool 15s+15s (<1.32 log remaining, n=10)
Chemical Treatments	**#01** ASC Mist 10s (100%, n=5) **#04** ASC Mist 30s (93%, n=15) **#06** ASC Mist+Wait+Rinse 15s+5m+5s (80%, n=5)	**#37** ASC Mist+Wait+Rinse 10s+5m+5s (>1.82 log reduction at >99% confidence, n=10) **#01** ASC Mist 10s (>1.57 log reduction at >99% confidence, n=5) **#32** TSP Mist 30s (~1.37 log reduction at >99% confidence, n=15)	**#01** ASC Mist 10s (<0.43 log remaining, n=5) **#04** ASC Mist 30s (<0.45 log remaining, n=15) **#02** ASC Mist 15s (<0.67 log remaining, n=20)
Campylobacters on neck skin	***Rated by proportion of samples <LoD***	***Rated by Reduction***	**Rated by Final Levels Remaining**
Physical Treatments	**#12** CW Mist+Wait+Rinse 15s+5m+5s (20%, n=5) **#29** Stm+Cool 15s+15s (7%, n=15) No other <LoD samples for physical treatments	**#13** CW OzoneNozzles 15s 0.55 log reduction at <99% confidence, n=5) **#30** Dry Stm+Cool 15s+15s 0.55 log reduction at <99% confidence, n=10) **#28** Stm 15s 0.53 log reduction at <99% confidence, n=15)	**#28** Stm 15s (2.05 log remaining, n=15) **#29** Stm+Cool 15s+15s (<2.09 log remaining, n=15) **#12** CW Mist+Wait+Rinse 15s+5m+5s (<2.21 log remaining, n=5)
Chemical Treatments	**#32** TSP Mist 30s (73%, n=15) **#02** ASC Mist 15s (65%, n=20)	**#32** TSP Mist 30s (>2.41 log reduction at >99% confidence, n=15) **#04** ASC Mist 30s (>1.60 log reduction at >99% confidence, n=15)	**#32** TSP Mist 30s (<0.81 log remaining, n=15) **#04** ASC Mist 30s (<0.85 log remaining, n=15)

	#04 ASC Mist 30s (47%, n=15)	#02 ASC Mist 15s (>1.45 log reduction at >99% confidence, n=20)	#02 ASC Mist 15s (<0.96 log remaining, n=20)
Enterobacteriaceae on breast skin	***Rated by proportion of samples <LoD***	***Rated by Reduction***	**Rated by Final Levels Remaining**
Physical Treatments	**#26** (no Stm+) Cool Only (0s+)15s (21%, n=15) **#19** HW Wash 2.5s (20%, n=5) **#29** Stm+Cool 15s+15s (13%, n=15)	**#29** Stm+Cool 15s+15s (>1.41 log reduction at >99% confidence, n=15) #28 Stm 15s (>1.02 log reduction at <99% confidence, n=15) **#30** Dry Stm+Cool 15s+15s (0.98 log reduction at <99% confidence, n=10)	**#29** Stm+Cool 15s+15s (<1.87 log remaining, n=15) **#19** HW Wash 2.5s (<1.88 log remaining, n=5) **#30** Dry Stm+Cool 15s+15s (2.12 log remaining, n=10)
Chemical Treatments	**#04** ASC Mist 30s (47%, n=15) **#02** ASC Mist 15s (45%, n=20) **#01** ASC Mist 10s (40%, n=5)	**#01** ASC Mist 10s (>2.40 log reduction at >99% confidence, n=5) **#04** ASC Mist 30s (>2.10 log reduction at >99% confidence, n=15) **#02** ASC Mist 15s (>1.58 log reduction at >99% confidence, n=20)	**#01** ASC Mist 10s (<0.61 log remaining, n=5) **#04** ASC Mist 30s (<1.17 log remaining, n=15) **#02** ASC Mist 15s (<1.31 log remaining, n=20)
***Enterobacteriaceae* on neck skin**	***Rated by proportion of samples <LoD***	***Rated by Reduction***	**Rated by Final Levels Remaining**
Physical Treatments	No samples < LoD	**#11** CW Mist 30s (0.38 log reduction at <99% confidence, n=55) **#30** Dry Stm+Cool 15s+15s (0.23 log reduction at <99% confidence, n=10) **#29** Stm+Cool 15s+15s (0.11 log reduction at <99% confidence, n=15)	**#16** CW Wash 2.5s (2.91 log remaining, n=10) **#12** CW Mist+Wait+Rinse 15s+5m+5s (3.11 log remaining, n=5) **#19** HW Wash 2.5s (3.21 log remaining, n=5)
Chemical Treatments	**#32** TSP Mist 30s (67%, n=15)	**#32** TSP Mist 30s (>3.29 log reduction at >99% confidence, n=15)	**#32** TSP Mist 30s (<0.88 log remaining, n=15)

	#01 ASC Mist 10s (20%, n=5) **#02** ASC Mist 15s (15%, n=20)	**#01** ASC Mist 10s (>2.18 log reduction at >99% confidence, n=5) **#04** ASC Mist 30s (>1.89 log reduction at >99% confidence, n=15)	**#01** ASC Mist 10s (<1.41 log remaining, n=5) **#02** ASC Mist 15s (<1.81 log remaining, n=20)
Pseudomonads on breast skin	***Rated by proportion of samples <LoD***	***Rated by Reduction***	**Rated by Final Levels Remaining**
Physical Treatments	**#28** Stm 15s (40%, n=15) **#26** (no Stm+) Cool Only (0s+)15s (33%, n=15) **#29** Stm+Cool 15s+15s (20%, n=15),	**#15** CW OzoneNozzles 3m (1.54 log reduction at <99% confidence, n=5 **#28** Stm 15s (>1.32 log reduction at <99% confidence, n=15) **#27** Stm 10s (1.06 log reduction at <99% confidence, n=5)	**#28** Stm 15s (<1.14 log remaining, n=15) **#27** Stm 10s (1.33 log remaining, n=5) **#15** CW OzoneNozzles 3m (1.45 log remaining, n=5
Chemical Treatments	**#01** ASC Mist 10s (40%, n=5) **#32** TSP Mist 30s (40%, n=15) **#02** ASC Mist 15s (25%, n=20)	**#01** ASC Mist 10s (>2.00 log reduction at >99% confidence, n=5) **#04** ASC Mist 30s (>1.21 log reduction at >99% confidence, n=15) **#02** ASC Mist 15s (>1.16 log reduction at >99% confidence, n=20)	**#32** TSP Mist 30s (<1.23 log remaining, n=15) **#01** ASC Mist 10s (<1.29 log remaining, n=5) **#02** ASC Mist 15s (<1.33 log remaining, n=20)
Pseudomonads on neck skin	***Rated by proportion of samples <LoD***	***Rated by Reduction***	**Rated by Final Levels Remaining**
Physical Treatments	No physical treatment samples <LoD	**#15** CW OzoneNozzles 3m (1.13 log reduction at >99% confidence, n=5) **#28** Stm 15s (0.42 log reduction at <99% confidence, n=15) **#27** Stm 10s (0.37 log reduction at <99% confidence, n=5)	**#28** Stm 15s (2.24 log remaining, n=15) **#15** CW OzoneNozzles 3m (2.25 log remaining, n=5 **#27** Stm 10s (2.33 log remaining, n=5)

Chemical Treatments	**#32** TSP Mist 30s (53%, n=15) **#31** TSP Mist 15s (10%, n=10) **#38** ASC Mist+Wait+Rinse 10s+30m+5s (10%, n=10)	**#32** TSP Mist 30s (>2.01 log reduction at >99% confidence, n=15) **#01** ASC Mist 10s (1.13 log reduction at >99% confidence, n=5) **#38** ASC Mist+Wait+Rinse 10s+30m+5s (>1.12 log reduction at >99% confidence, n=10)	**#32** TSP Mist 30s (<0.81 log remaining, n=15) **#34** TSP Mist+Rinse 30s+30s (1.53 log remaining, n=5) **#33** TSP Mist+Rinse 15s+30s (1.59 log remaining, n=5)

Applying a scoring scheme where most effective treatment in a carcass part / organism / ranking method category scores 3 points, second most effective 2 points and third most effective 1 point, the overall ranking of treatments is shown in Table 24.

Table 24. Overall ranking of antimicrobial efficacy across all carcass part / organism categories (pts = points, other abbreviations are in Table 16)

Physical	Chemical
#28 Stm Stm 15s (27 pts)	#32 TSP Mist 30s (32pts)
#29 Stm Stm+Cool 15s+15s (20 pts)	#01 ASC Mist 10s (31pts)
#15 CW OzoneNozzles 3m (9 pts)	#04 ASC Mist 30s (19pts)
#30 Stm-Dry Stm+Cool 15s+15s (8 pts)	#02 ASC Mist 15s (14pts)
#12 CW Mist+Wait+Rinse 15s+5m+5s (6 pts)	#37 ASC Mist+Wait+Rinse 10s+5m+5s (3pts)
#26 (no Stm) Cool 15s Only (6 pts)	#34 TSP Mist+Rinse 30s+30s (2 pts)
#19 HW Wash 2.5s (5 pts)	#38 ASC Mist+Wait+Rinse 10s+30m+5s (2pts)
#27 Stm Stm 10s (5 pts)	#06 ASC Mist+Wait+Rinse 15s+5m+5s (1pt)
#11 CW Mist 30s (3 pts)	#31 TSP Mist 15s (1pt)
#13 CW OzoneNozzles 15s (3 pts)	#33 TSP Mist+Rinse 15s+30s (1 pt)
#16 CW Wash 2.5s (3 pts)	

Further data analysis in this report is therefore focussed on steam and cold-water sprays for physical treatments, and ASC and TSP for chemical treatments.

Two durations are pertinent for chemical treatments; the time for which the carcass is in the chemical application spray, and for rinsed carcasses, the wait (dwell) time for chemical action to occur between end of chemical application spray and rinsing spray.

10.3 Results: Evaluation by Carcass Part / Organism

10.3.1 Campylobacter on breast skin (see Table 17)

10.3.1.1 ASC

A summary of all ASC treatments for Campylobacter on breast skin is given in Table 25.

Table 25. Summary of effect of ASC treatment on Campylobacter on breast skin (see Table 16 for abbreviations)

Treatment (s = seconds)	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#01 ASC Mist 10 s	<0.43	0.26	>1.57	Y	#36, #38, #39
#02 ASC Mist 15 s	<0.67	0.61	~0.85	Y	#39
#04 ASC Mist 30 s	<0.45	0.17	>1.28	Y	#36, #38, #39
#05 ASC Mist+Wait+Rinse 15s+30s+30 s	<1.34	0.66	~-0.37	n	
#06 ASC Mist+Wait+Rinse 15s+5m+5s	<0.72	0.68	>0.52	n	
#36 ASC Mist+Rinse 10s+5s	<1.39	0.79	>1.26	Y	#01, #04
#37 ASC Mist+Wait+Rinse 10s+5m+5s	<0.86	0.62	>1.82	Y	#39
#38 ASC Mist+Wait+Rinse 10s+30m+5s	<1.38	0.65	>1.27	Y	#01, #04
#39 ASC Mist+Wait+Rinse 10s+60m+5s	1.70	0.63	0.95	Y	#01, #02, #04, #37

**All permutations of final levels are not significantly different except pairings indicated.*

10.3.1.1.1 Application Time

Treatments #01, #02 and #04 for 10, 15 and 30 seconds misting spray with no rinsing gave reductions of >1.57, ~0.85, and >1.28 $\log_{10}$ cfu g^{-1} respectively. All gave significant reductions from control levels, however, the results showed no trend with increasing treatment time and were not statistically different from each other.

10.3.1.1.2 Effects of Rinsing

Treatment #05 was a repeat of the treatment #02 (15 seconds mist) with 30 seconds chemical action time and 30 seconds rinse designed to give good wash off performance. An increase of ~0.37 $\log_{10}$ cfu g^{-1} was observed and final levels were 0.67 $\log_{10}$ cfu g^{-1} higher than the 30 seconds CW mist rinse control (treatment #11). There was no significant effect from control levels.

Treatment #06 with a longer action time and shorter rinse gave a reduction of >0.52 $\log_{10}$ cfu g^{-1}, but no significant change from untreated control levels. Thus, rinsing appeared to negate the decontamination effect.

10.3.1.1.3 Chemical Action (Wait) Time

Treatments #36 to #39 investigated the effect of increasing the chemical action wait (dwell) time. All treatments gave significant reductions from control levels, however, were not statistically different from each other. Reductions were broadly equivalent to the unrinsed 10 seconds treatment (#01). There is possibly a slight trend towards an increased final level after treatment and decreased reduction with increasing chemical action time, but correlations are low (Figure 29).

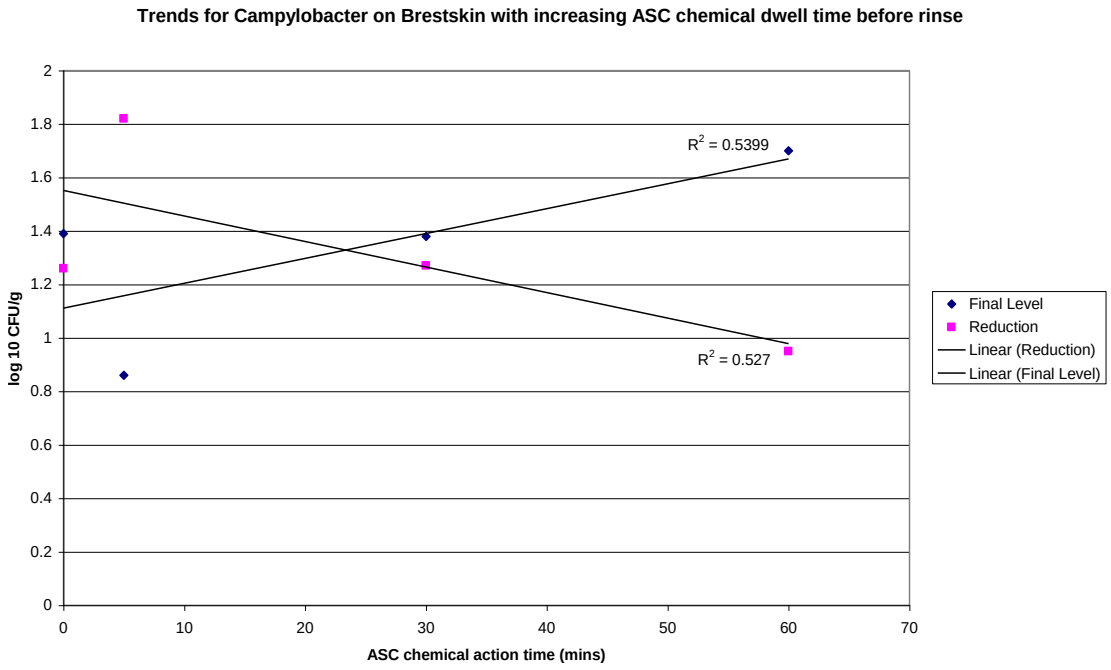


Figure 29. Trends for Campylobacter on breast skin for increasing ASC dwell time before rinse

10.3.1.2 TSP

A summary of all TSP treatments for Campylobacter on breast skin is given in Table 26.

Table 26. Summary of effects of TSP treatments of campylobacters on breast skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#31 TSP Mist 15 seconds	1.92	0.61	0.58	Y	#32
#32 TSP Mist 30 seconds	<0.79	0.38	~1.37	Y	#31, #33, #34
#33 TSP Mist+Rinse 15 seconds+30 seconds	1.79	0.35	0.58	Y	#32
#34 TSP Mist+Rinse 30 seconds+30 seconds	1.50	0.28	0.87	Y	#32

**All permutations of final levels are not significantly different except pairings indicated.*

10.3.1.2.1 Effects of Application Time

Treatments #31 and #32 for 15 and 30 seconds misting spray with no rinsing gave reductions of 0.58, and ~1.37 $\log_{10}$ cfu g^{-1} respectively. These treatments both gave significantly different reductions from control levels and were significantly different from each other, with a trend for increasing reduction with longer spraying times.

Treatments #33 and #34 for 15 and 30 seconds misting spray with rinsing gave lower reductions of 0.58, and 0.87 $\log_{10}$ cfu g^{-1}, respectively. These treatments also both gave significant reductions from control levels, with a trend for increasing reduction with longer spraying times, but unlike the non-rinsed treatments were not significantly different from each other.

In both rinsed and non-rinsed treatment groups there was a trend for increasing efficacy with longer spray application durations.

10.3.1.2.2 Effects of Rinsing

Rinsing treatments #33 and #34 gave significant reductions from control levels, and were not different from each other. When compared to the non-rinsed treatments #31 and #32 it is seen that rinsing reduces effects of TSC. The trend for increased reductions with increased spray time as seen for non-rinsed treatments was preserved but at a lower magnitude.

10.3.1.3 Steam

A summary of all steam treatments for Campylobacter on breast skin is given in Table 27.

Table 27. Summary of effect of steam treatments on Campylobacters on breast skin

Treatment(s = seconds)	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#26 (no Stm) Cool 15s Only	<1.49	0.95	~0.66	n	
#27 Stm Stm 10s	1.50	0.45	-0.19	n	
#28 Stm Stm 15s	<0.87	0.63	~1.28	Y	
#29 Stm Stm+Cool 15s+15s	<0.91	0.55	~1.24	Y	
#30 Stm-Dry Stm+Cool 15s+15s	<1.32	1.04	>0.81	n	

**All permutations of final levels are not significantly different.*

10.3.1.3.1 Application Time

Increasing the steam treatment from 10 seconds (#27) to 15 seconds (#28), increased reductions to a significant level, although there were no significant differences in the final levels between both treatment groups.

10.3.1.3.2 Effects of Cooling Spray

Adding a 15 seconds cooling spray after the 15 seconds steam treatment (#29) made no further improvement to final levels nor reductions seen from steam alone.

10.3.1.4 Cold Water Spray Configuration

A summary of all cold water treatments with various spray configurations for Campylobacter on breast skin is given in Table 28

Table 28. Summary of effect of Cold Water Spray Configurations on Campylobacters on breast skin

Treatment (s = seconds; m = minutes)	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#10 CW Mist 15s	<2.25	0.73	~0.03	n	#13
#11 CW Mist 30s	1.93	0.52	<0.19	n	
#12 CW Mist+Wait+Rinse 15s+5m+5s	1.60	0.42	-0.36	n	
#13 CW OzoneNozzles 15s	1.60	0.33	0.68	Y	#10
#14 CW OzoneNozzles 30s	2.02	0.33	0.26	n	
#15 CW OzoneNozzles 3m	2.02	0.13	-0.30	n	
#16 CW Wash 2.5s	2.07	0.15	-0.22	n	
#26 (no Stm) Cool 15s Only	<1.49	0.95	~0.66	n	
#40 CW HighIntensity 5s	2.13	0.48	0.58	n	
#41 CW HighIntensity 15s	1.98	1.09	0.73	n	
#42 CW HighIntensity 30s	2.12	0.15	0.59	n	

**All permutations of final levels are not significantly different except pairing indicated.*

Only treatment #13 had a significant effect over control levels, however final levels were only significantly different to treatment #10. Cold water treatments were therefore mostly not effective.

10.3.2 Campylobacter on neck skin (see Table 18)

10.3.2.1 ASC

A summary of all ASC treatments for Campylobacter on neck skin is given in Table 29

Table 29. Summary of effect of ASC treatments on campylobacter on neck skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#01 ASC Mist 10s	<1.41	1.47	>1.45	n	
#02 ASC Mist 15s	<0.96	0.92	>1.45	Y	#38
#04 ASC Mist 30s	<0.85	0.60	>1.60	Y	#36, #37, #38
#05 ASC Mist+Wait+Rinse 15s+30s+30s	<1.48	0.89	>0.39	n	
#06 ASC Mist+Wait+Rinse 15s+5m+5s	<1.55	0.89	>0.85	n	#38
#36 ASC Mist+Rinse 10s+5s	<1.80	0.73	>1.08	Y	#04
#37 ASC Mist+Wait+Rinse 10s+5m+5s	<1.76	0.61	>1.12	Y	#04
#38 ASC Mist+Wait+Rinse 10s+30m+5s	2.33	0.54	0.55	n	#02, #04, #06
#39 ASC Mist+Wait+Rinse 10s+60m+5s	<1.89	1.07	>0.99	n	

**All permutations of final levels are not significantly different except pairings indicated.*

10.3.2.1.1 Application Time

Treatments #01, #02 and #04 for 10, 15 and 30 seconds misting spray with no rinsing gave final levels of <1.41, <0.96 and <0.85 $\log_{10}$ cfu g^{-1}, respectively. This showed a trend of

decreasing final levels with increasing application duration. However, this trend was only partially carried over into the reductions. The variability of results as indicated by the standard deviations was reduced by increasing the treatment time (Figure 30).

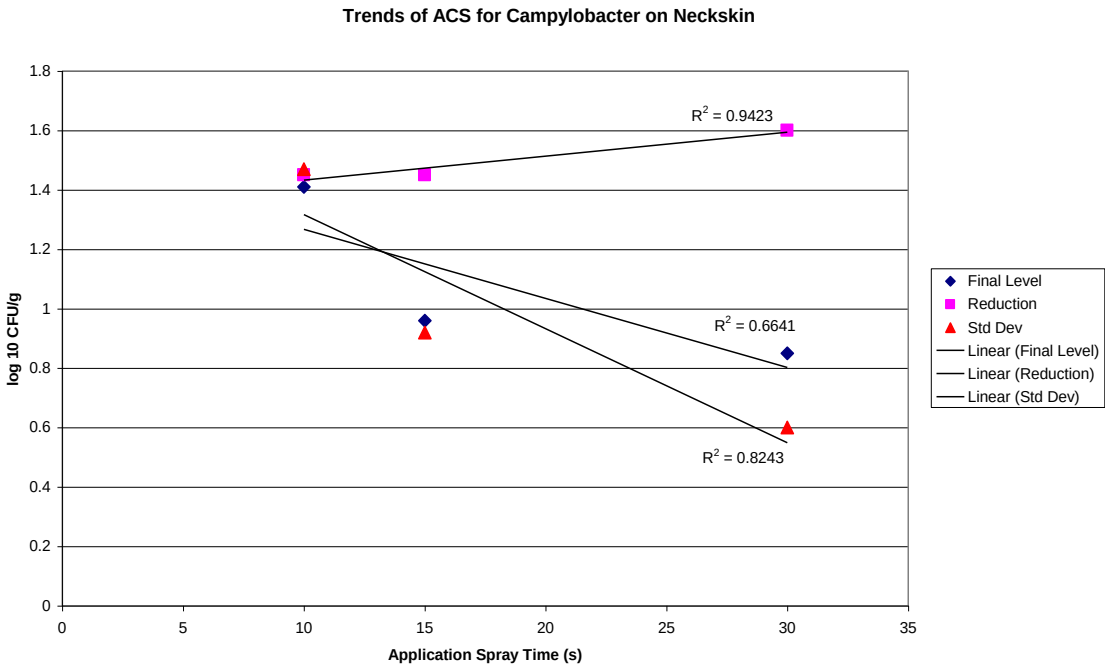


Figure 30. Effect of increasing ASC application time on reductions achieved and on standard deviation for Campylobacter on neck skin

The higher SD for the 10 seconds (#01) treatment probably accounts for it not producing significant reductions from control levels, however, all non-rinsed treated results were not statistically different from each other.

10.3.2.1.2 Effects of Rinsing

Treatment #05 was a repeat of the treatment #02 (15 second mist) with 30 seconds chemical action time before a 30 seconds rinse designed to give a good wash-off performance. A decreased reduction to 0.39 $\log_{10}$ cfu g^{-1} was observed and final levels rose by 0.52 $\log_{10}$ cfu g^{-1}. Adding the rinse made the 15 seconds ASC treatment not significantly different from the control levels.

Treatment #06 with a longer action time and shorter rinse gave even higher final levels, yet an improved reduction of >0.85 $\log_{10}$ cfu g^{-1}, but was still not a significant change from untreated control levels.

10.3.2.1.3 Chemical Action (Wait) Time

Treatments #36 to #39 investigated the effect of increasing the chemical action wait time. The shorter wait treatments gave significant reductions from control levels; however, there were no obvious trends with increasing chemical action dwell time.

10.3.2.2 TSP

A summary of all TSP treatments for Campylobacter on neck skin is given in Table 30.

Table 30. Summary of effect of TSP treatments on Campylobacter on neck skin

Treatment (s = seconds)	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#31 TSP Mist 15s	<1.89	1.22	>1.36	Y	
#32 TSP Mist 30s	<0.81	0.74	>2.41	Y	#33, #34
#33 TSP Mist+Rinse 15s+30s	2.25	0.42	0.99	Y	#32
#34 TSP Mist+Rinse 30s+30s	2.11	0.52	1.13	Y	#32

**All permutations of final levels are not significantly different except pairings indicated.*

10.3.2.2.1 Effects of Application Time

Treatments #31 and #32 for 15 and 30 seconds misting spray with no rinsing gave good reductions of >1.36, and >2.41 $\log_{10}$ cfu g^{-1}, respectively. The two treatments showed a trend for increasing reduction and decreasing final levels with longer spraying times, and gave significant changes from control levels, however they were not significantly different from each other.

10.3.2.2.2 Effects of Rinsing

Rinsed treatments #33 and #34 both gave significant reductions from control levels, and were not different from each other. When compared to the non-rinsed treatments #31 and #32 it is seen that rinsing reduces the effectiveness of TSP. The trend for increased reductions with increased spray time as seen for non-rinsed treatments was not evident for results when rinsing was used.

10.3.2.3 Steam

A summary of all steam treatments for Campylobacter on neck skin is given in Table 31.

Table 31. Summary of effect of steam treatments on Campylobacter on neck skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#26 (no Stm) Cool 15s Only	2.49	1.01	0.09	n	
#27 Stm Stm 10s	2.35	0.22	-0.78	Y	
#28 Stm Stm 15s	2.05	0.85	0.53	n	
#29 Stm Stm+Cool 15s+15s	<2.09	1.03	>0.49	n	
#30 Stm-Dry Stm+Cool 15s+15s	2.64	0.97	0.55	n	

**All permutations of final levels are not significantly different.*

10.3.2.3.1 Application Time

There were no significant differences in the final levels of Campylobacter on the neck skin for all steam treatments. Increasing the steam treatment from 10 seconds (#27) to 15 seconds (#28) decreased final levels, and improved reductions. However, the 10 second treatment (#27) showed a significant increase of Campylobacters between control and treated.

10.3.2.3.2 Effects of Cooling Spray

Adding a 15 seconds cooling spray after the 15 seconds steam treatment (#29) made no substantial further improvement to final levels nor reductions seen from steam alone.

10.3.2.4 Cold Water Spray Configuration

A summary of all cold water treatments with various spray configurations for Campylobacter on neck skin is given in Table 32.

Table 32. Summary of effect of Cold Water Spray Configurations on Campylobacter on neck skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#10 CW Mist 15s	2.78	0.84	0.38	n	
#11 CW Mist 30s	2.41	0.61	0.03	Y	
#12 CW Mist+Wait+Rinse 15s+5m+5s	<2.21	1.17	>0.19	n	
#13 CW OzoneNozzles 15s	2.43	0.04	0.55	n	
#14 CW OzoneNozzles 30s	2.91	0.60	0.07	n	
#15 CW OzoneNozzles 3m	3.02	0.45	-1.04	Y	
#16 CW Wash 2.5s	2.72	0.78	-0.26	n	
#26 (no Stm) Cool 15s Only	2.49	1.01	0.09	n	
#40 CW HighIntensity 5s	2.91	0.49	0.14	n	
#41 CW HighIntensity 15s	2.68	0.40	0.37	n	
#42 CW HighIntensity 30s	2.93	0.49	0.12	n	

**All permutations of final levels are not significantly different.*

Only treatments #11 and #15 showed significant changes from control levels. Although small, the large number of replicates (n=55) produced the significant difference seen for #11, and #15 actually gave a significant *increase* in levels between controls and treated. There were no significant differences in final levels across all cold water spray configurations evaluated.

10.3.3 Enterobacteriaceae on breast skin (see Table 19)

10.3.3.1 ASC

A summary of all ASC treatments for Enterobacteriaceae on breast skin is given in Table 33.

Table 33. Summary of effect of ASC treatment for Enterobacteriaceae on breast skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#01 ASC Mist 10s	<0.61	0.32	>2.40	Y	#05, #06, #36, #37, #38, #39
#02 ASC Mist 15s	<1.31	0.87	>1.58	Y	#05, #36, #37, #38, #39
#04 ASC Mist 30s	<1.17	0.87	>2.10	Y	#05, #36, #37, #38, #39
#05 ASC Mist+Wait+Rinse 15s+30s+30s	2.47	0.34	-0.08	n	#01, #02, #04, #37
#06 ASC Mist+Wait+Rinse 15s+5m+5s	1.99	0.39	0.80	n	#01, #36, #37, #38, #39
#36 ASC Mist+Rinse 10s+5s	3.14	0.62	0.56	n	#01, #02, #04, #06
#37 ASC Mist+Wait+Rinse 10s+5m+5s	3.53	0.36	0.17	n	#01, #02, #04, #05, #06
#38 ASC Mist+Wait+Rinse 10s+30m+5s	2.92	0.69	0.78	n	#01, #02, #04, #06
#39 ASC Mist+Wait+Rinse 10s+60m+5s	3.11	0.59	0.59	n	#01, #02, #04, #06

**All permutations of final levels are not significantly different except pairings indicated.*

10.3.3.1.1 Application Time

Treatments #01, #02 and #04 for 10, 15 and 30 seconds (misting spray with no rinsing) gave reductions of >2.40, >2.10, and >1.58 $\log_{10}$ cfu g^{-1} respectively. All gave significant reductions from control levels, however, the treated results showed no trend with increasing duration and were not statistically different from each other.

10.3.3.1.2 Effects of Rinsing

Treatment #05 was a repeat of the treatment #02 (15 seconds mist) with 30 seconds chemical action time and 30 seconds rinse, designed to give good wash off performance. A slight increase of 0.08 $\log_{10}$ cfu g^{-1} was observed. This negligible effect was in keeping with the small 0.13 $\log_{10}$ cfu g^{-1} reduction seen for the 30 seconds CW mist rinse control (treatment #11).

Treatment #06 with a longer action time and shorter rinse gave a reduction of 0.8 $\log_{10}$ cfu g^{-1}, but no significant change from untreated control levels.

10.3.3.1.3 Chemical Action (Wait) Time

Treatments #36 to #39 investigated the effect of increasing the chemical action wait (dwell) time to 30 or 60 minutes before rinsing. There were no apparent trends with increasing wait time, nor were reductions increased over those observed after 5 minutes dwell time and results #36 to #39 were not significantly different.

10.3.3.2 TSP

A summary of all TSP treatments for Enterobacteriaceae on breast skin is given in Table 34.

Table 34. Summary of effect of TSP treatments on Enterobacteriaceae on breast skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#31 TSP Mist 15s	3.49	0.89	0.89	n	#32
#32 TSP Mist 30s	<2.13	1.26	>1.26	n	#31
#33 TSP Mist+Rinse 15s+30s	2.73	0.27	0.27	n	
#34 TSP Mist+Rinse 30s+30s	2.80	0.27	0.27	n	

**All permutations of final levels are not significantly different except pairing indicated.*

10.3.3.2.1 Effects of Application Time

Treatments #31 and #32 for 15 and 30 seconds misting spray with no rinsing gave reductions of 0.89, and >1.26 $\log_{10}$ cfu g^{-1}, respectively. Whilst these values were significantly different from each other, with a trend for increasing reduction with longer spraying times neither treatment gave significantly different reduction from control levels.

10.3.3.2.2 Effects of Rinsing

Rinsed treatments #33 and #34 gave low, non-significant reductions from control levels, and were not different from each other. Rinsing negated the effect of TSP, irrespective of spray time. The trend for increased reductions with increased spray time was not preserved.

10.3.3.3 Steam

A summary of all steam treatments for Enterobacteriaceae on breast skin is given in Table 35.

Table 35. Summary of Steam treatments of Enterobacteriaceae on breast skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#26 (no Stm) Cool 15s Only	<2.53	1.27	>0.75	n	
#27 Stm Stm 10s	2.88	0.31	0.30	n	#29
#28 Stm Stm 15s	<2.26	0.99	>1.02	Y	
#29 Stm Stm+Cool 15s+15s	<1.87	0.88	>1.41	Y	#27
#30 Stm-Dry Stm+Cool 15s+15s	2.12	0.91	0.98	n	

**All permutations of final levels are not significantly different except pairing indicated.*

10.3.3.3.1 Application Time

Increasing steam duration from 10 seconds (#27) to 15 seconds (#28) improved reductions from <0.30 to 1.02 $\log_{10}$ cfu g^{-1} with the latter being a significant effect.

10.3.3.3.2 Effects of Cooling Spray

Adding a 15 seconds cooling spray after the 15 seconds steam treatment (#29) gave an increased significant reduction of <1.41 $\log_{10}$ cfu g^{-1}.

10.3.3.4 Cold Water Spray Configuration

A summary of all cold water treatments with various spray configurations for Enterobacteriaceae on breast skin is given in Table 36

Table 36. Summary of effect of Cold Water Spray Configurations on *Enterobacteriaceae* on breast skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#10 CW Mist 15s	3.53	0.73	-0.23	n	#15, #16
#11 CW Mist 30s	3.66	0.88	0.13	n	#14, #15, #16, #26, #40
#12 CW Mist+Wait+Rinse 15s+5m+5s	3.18	0.39	-0.39	n	#16
#13 CW OzoneNozzles 15s	3.33	0.21	-0.47	n	#16
#14 CW OzoneNozzles 30s	3.02	0.37	-0.16	n	#11, #16
#15 CW OzoneNozzles 3m	2.66	0.52	0.40	n	#10, #11
#16 CW Wash 2.5s	2.16	0.19	-0.13	n	#10, #11, #12, #13, #14, #40, #41, #42
#26 (no Stm) Cool 15s Only	<2.53	1.27	>0.75	n	#11
#40 CW HighIntensity 5s	3.19	0.17	0.07	n	#11, #16
#41 CW HighIntensity 15s	3.43	0.49	-0.17	n	#16
#42 CW HighIntensity 30s	3.46	0.25	-0.20	n	#16

**All permutations of final levels are not significantly different except pairings indicated.*

None of the cold water spray configurations had a significant effect as treatments, and all reductions (except #26) are relatively small. Since most treatment effects are small, this accounts for the high degree of similarity between the various spray configurations, and no detectable trends with increasing spray durations.

10.3.4 Enterobacteriaceae on neck skin (see Table 20)

10.3.4.1 ASC

A summary of all ASC treatments for Enterobacteriaceae on neck skin is given in Table 37

Table 37. Summary of effect of ASC treatments on Enterobacteriaceae on the neck skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#01 ASC Mist 10s	<1.41	0.87	>2.18	Y	#36, #37, #38, #39
#02 ASC Mist 15s	<1.81	1.00	>1.77	Y	#06, #36, #37, #38, #39
#04 ASC Mist 30s	<2.07	0.80	>1.89	Y	#36, #37, #38, #39
#05 ASC Mist+Wait+Rinse 15s+30s+30s	2.23	0.55	0.91	n	#36, #37, #38
#06 ASC Mist+Wait+Rinse 15s+5m+5s	2.78	0.32	0.42	n	#02, #37
#36 ASC Mist+Rinse 10s+5s	3.30	0.45	0.71	n	#01, #02, #04, #05
#37 ASC Mist+Wait+Rinse 10s+5m+5s	3.80	0.43	0.21	n	#01, #02, #04, #05, #06
#38 ASC Mist+Wait+Rinse 10s+30m+5s	3.37	0.69	0.64	n	#01, #02, #04, #05
#39 ASC Mist+Wait+Rinse 10s+60m+5s	3.32	0.76	0.69	n	#01, #02, #04

**All permutations of final levels are not significantly different except pairings indicated.*

10.3.4.1.1 Application Time

Treatments #01, #02 and #04 for 10, 15 and 30 seconds misting spray with no rinsing gave final levels of <1.41, <1.81 and <2.07 log $_{10}$ cfu g^{-1}, respectively. This trend was the reverse of that intuitive expectation of reducing final levels with increasing treatment time. The trend is not explained by either numbers of counts below LoD or control levels (Table 38).

Table 38. Controls and <LoD for treatments #01, #02 and #04

	Treateted samples			Control samples		
Treatment	Mean after ($\log_{10}$ cfu g^{-1})	SD	% <LoD	Mean before ($\log_{10}$ cfu g^{-1})	SD	% <LoD
#01 ASC Mist 10s	<1.41	0.87	20%	3.59	0.83	0%
#02 ASC Mist 15s	<1.81	1.00	15%	3.58	0.67	0%
#04 ASC Mist 30s	<2.07	0.80	7%	3.96	0.64	0%

However, since all values are '<' inequalities the true relationship is not discernable. Despite this trend, all treatments were significant and gave good reductions of around 2 $\log_{10}$ cfu g^{-1}.

10.3.4.1.2 Effects of Rinsing

Treatment #05 was a repeat of the treatment #02 (15 second mist) with 30 seconds chemical action time before a 30 seconds rinse designed to give good wash-off performance. A decreased reduction to 0.91 $\log_{10}$ cfu g^{-1} was observed and final levels rose by 0.42 $\log_{10}$ cfu g^{-1}. Adding the rinse made the 15 seconds ASC treatment not significantly different from the control levels.

Treatment #06 with a longer action time and shorter rinse gave even higher final levels, and reduced reduction of 0.42 $\log_{10}$ cfu g^{-1}, that was not a significant change from untreated control levels.

10.3.4.1.3 Chemical Action (Wait) Time

Treatments #36 to #39 investigated the effect of increasing the chemical action wait time. No treatments gave significant reductions from control levels; and there were no obvious trends with increasing chemical action wait time.

10.3.4.2 TSP

A summary of all TSP treatments for Enterobacteriaceae on neck skin is given in Table 39.

Table 39. Summary of TSP treatments of Enterobacteriaceae on neck skin

Treatment	Mean final level after treatment (log $_{10}$ cfu g^{-1})	SD	Reduction (log $_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#31 TSP Mist 15s	2.59	1.62	1.70	n	#32
#32 TSP Mist 30s	<0.88	0.83	>3.29	Y	#31, #33, #34
#33 TSP Mist+Rinse 15s+30s	2.86	0.48	0.48	n	#32
#34 TSP Mist+Rinse 30s+30s	2.89	0.22	0.45	n	#32

**All permutations of final levels are not significantly different except pairings indicated.*

10.3.4.2.1 Effects of Application Time

Treatments #31 and #32 for 15 and 30 seconds misting spray with no rinsing gave good reductions of >1.70, and >3.29 $\log_{10}$ cfu g^{-1}, respectively. The two treatments showed a trend for increasing reduction and decreasing final levels with longer spraying times. However only treatment #32 (30seconds) gave a significant reduction from control levels.

10.3.4.2.2 Effects of Rinsing

When rinsed treatments #33 and #34 are compared to the non-rinsed treatments #31 and #32 it is seen that rinsing substantially reduces the effects of TSP to around 0.5 log_{10} cfu g^{-1} irrespective of spray application time.

10.3.4.3 Steam

A summary of all steam treatments for Enterobacteriaceae on neck skin is given in Table 40.

Table 40. Summary of effect of Steam treatments on Enterobacteriaceae on neck skin

Treatment	Mean final level after treatment (log_{10} cfu g^{-1})	SD	Reduction (log_{10} cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#26 (no Stm) Cool 15s Only	3.78	0.61	-0.06	n	
#27 Stm Stm 10s	3.71	0.74	-0.01	n	
#28 Stm Stm 15s	3.70	0.57	0.02	n	
#29 Stm Stm+Cool 15s+15s	3.61	0.63	0.11	n	
#30 Stm-Dry Stm+Cool 15s+15s	3.65	0.79	0.23	n	

**All permutations of final levels are not significantly different.*

10.3.4.3.1 Application Time

All steam treatments gave negligible reductions compared to control levels for Enterobacteriaceae on the neck skin. There were no significant effects of any treatment, and no significant differences between the final levels of for all treatments.

10.3.4.3.2 Effects of Cooling Spray.

Adding a 15 seconds cooling spray after the 15 seconds steam treatment (#29) made a slight improvement (not significant) to reductions, however effects are still very small.

10.3.4.4 Cold Water Spray Configuration

A summary of all cold water treatments with various spray configurations for Enterobacteriaceae on neck skin is given in Table 41.

Table 41. Summary of effect of Cold Water Spray Configurations on Enterobacteriaceae on neck skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#10 CW Mist 15s	3.94	0.58	0.07	n	#12, #15, #16
#11 CW Mist 30s	3.78	0.79	0.38	n	#16
#12 CW Mist+Wait+Rinse 15s+5m+5s	3.11	0.51	0.09	n	#10
#13 CW OzoneNozzles 15s	3.69	0.32	-0.14	n	#16
#14 CW OzoneNozzles 30s	3.72	0.40	-0.17	n	#16
#15 CW OzoneNozzles 3m	3.41	0.31	-0.05	n	#10
#16 CW Wash 2.5s	2.91	0.44	-0.07	N	#10, #11, #13, #14, #26, #42
#26 (no Stm) Cool 15s Only	3.78	0.61	-0.06	N	#16
#40 CW HighIntensity 5s	3.79	0.60	-0.14	N	
#41 CW HighIntensity 15s	3.64	0.46	0.01	N	
#42 CW HighIntensity 30s	3.85	0.24	-0.20	N	#16

**All permutations of final levels are not significantly different except pairings indicated.*

No coldwater treatments gave significant reductions in Enterobacteriaceae on neck skin compared to control levels. In all cases reductions were low or showed apparent increases in Enterobacteriaceae levels after treatment.

10.3.5 Pseudomonads on breast skin (see Table 21)

10.3.5.1 ASC

A summary of all ASC treatments for Pseudomonads on breast skin is given in Table 42.

Table 42. Summary of effect of ASC treatments on *Pseudomonas* on breast skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#01 ASC Mist 10s	<1.29	0.78	>2.00	Y	#36
#02 ASC Mist 15s	<1.33	0.70	>1.16	Y	#36, #37
#04 ASC Mist 30s	<1.57	0.71	>1.21	Y	#36, #37
#05 ASC Mist+Wait+Rinse 15s+30s+30s	1.56	0.40	0.13	N	#36
#06 ASC Mist+Wait+Rinse 15s+5m+5s	<1.59	0.81	>0.96	N	
#36 ASC Mist+Rinse 10s+5s	2.70	0.46	0.29	N	#01, #02, #04, #05
#37 ASC Mist+Wait+Rinse 10s+5m+5s	2.33	0.55	0.66	N	#02, #04
#38 ASC Mist+Wait+Rinse 10s+30m+5s	2.00	0.70	0.99	N	
#39 ASC Mist+Wait+Rinse 10s+60m+5s	2.08	0.75	0.91	N	

**All permutations of final levels are not significantly different except pairings indicated.*

10.3.5.1.1 Application Time

All non-rinsed treatments gave significant reductions over control levels, but were not significantly different from each other (Figure 31).

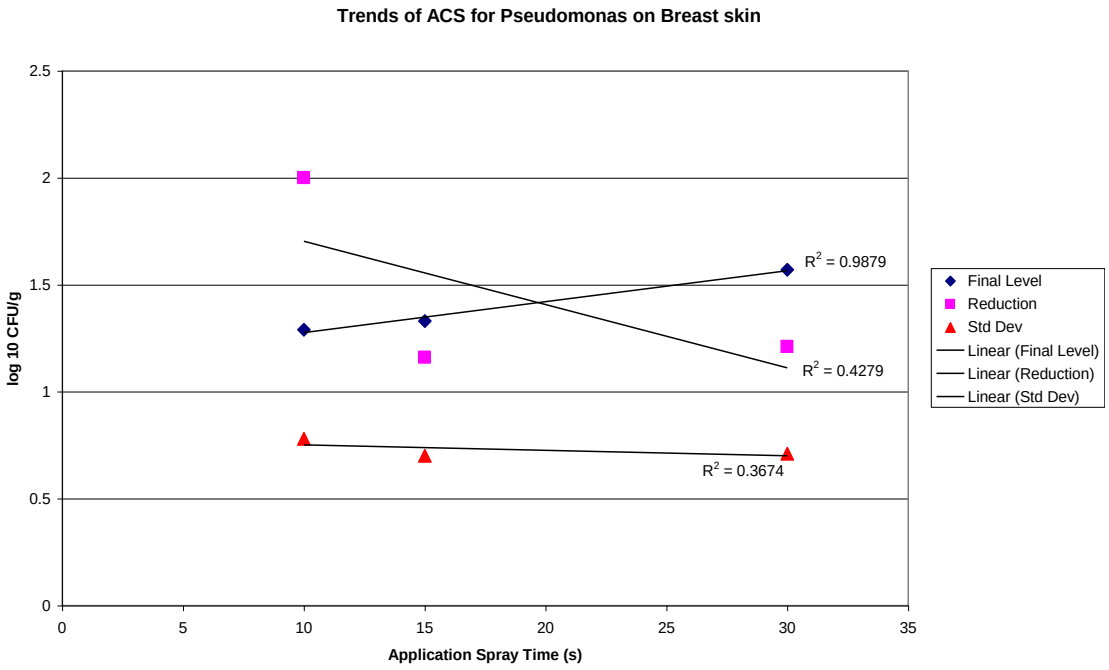


Figure 31. Trends of ASC for *Pseudomonas* on breast skin

10.3.5.1.2 Effects of Rinsing

Treatment #05 was a repeat of the treatment #02 (15 seconds mist) with 30 seconds chemical action time before a 30 seconds rinse designed to give good wash off performance. Adding the rinse substantially decreased the effectiveness of the 15 seconds ASC treatment, and gave final levels not significantly different from the control levels.

Treatment #06 with a longer action time and shorter rinse gave even higher final levels, yet an improved reduction of >0.96 $\log_{10}$ cfu g^{-1} over treatment #05, but was still not a significant improvement over untreated control levels.

10.3.5.1.3 Chemical Action (Wait) Time

Treatments #36 to #39 investigated the effect of increasing the chemical action wait time. None gave significant reductions from control levels and there were no obvious trends with increasing chemical action dwell time.

10.3.5.2 TSP

A summary of all TSP treatments for Pseudomonads on breast skin is given in Table 43.

Table 43. Summary of effect of TSP treatments on pseudomonas on breast skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#31 TSP Mist 15s	<2.02	0.90	>0.43	n	
#32 TSP Mist 30s	<1.23	0.76	~0.75	n	
#33 TSP Mist+Rinse 15s+30s	1.42	0.42	0.41	n	
#34 TSP Mist+Rinse 30s+30s	1.37	0.61	0.46	n	

**All permutations of final levels are not significantly different.*

10.3.5.2.1 Effects of Application Time

Treatments #31 and #32 for 15 and 30 seconds misting spray with no rinsing gave non-significant reductions of >0.43, and ~0.75 $\log_{10}$ cfu g^{-1} respectively. The two treatments showed a trend for increasing reduction and decreasing final levels with longer spraying times, but were not significantly different from each other.

10.3.5.2.2 Effects of Rinsing

Rinsed treatments #33 and #34 both gave non-significant reductions by comparison with control levels, and were not different from each other. When compared to the non-rinsed treatments #31 and #32 it is seen that rinsing reduces the effect of TSP.

10.3.5.3 Steam

A summary of all steam treatments for Pseudomonads on breast skin is given in Table 44.

Table 44. Summary of effect of Steam treatments on pseudomonads on breast skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#26 (no Stm) Cool 15s Only	<2.58	1.56	~-0.12	n	#28
#27 Stm Stm 10s	1.33	0.34	1.06	Y	#30
#28 Stm Stm 15s	<1.14	0.77	>1.32	Y	#26, #29, #30
#29 Stm Stm+Cool 15s+15s	<2.20	1.11	>0.26	n	#28
#30 Stm-Dry Stm+Cool 15s+15s	2.68	0.60	-0.41	n	#27, #28

**All permutations of final levels are not significantly different except pairings indicated.*

10.3.5.3.1 Application Time

Both 10 seconds (#27) and 15 seconds (#28) steam treatments gave significant reductions of Pseudomonads on breast skin. However, despite a trend for decreased final levels and improved reductions for increasing treatment duration, the two treatments were not significantly different from each other.

10.3.5.3.2 Effects of Cooling Spray

Adding a 15 second cooling spray after the 15 second steam treatment (#29) reduced the reduction seen from steam alone (#28), and was not significantly different from the cooling spray alone (#26).

10.3.5.4 Cold Water Spray Configuration

A summary of all cold water treatments with various spray configurations for Pseudomonads on breast skin is given in Table 45.

Table 45. Summary of Cold Water Spray Configurations of pseudomonads on breast skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#10 CW Mist 15s	2.42	0.64	-0.13	n	#11, #15, #16
#11 CW Mist 30s	3.07	0.95	<-0.29	n	#10, #12, #13, #14, #15, #16, #40, #41, #42
#12 CW Mist+Wait+Rinse 15s+5m+5s	2.55	0.06	0.00	n	#11, #15, #16
#13 CW OzoneNozzles 15s	2.38	0.27	-0.33	n	#11, #15, #16
#14 CW OzoneNozzles 30s	2.11	0.26	-0.06	n	#11, #16
#15 CW OzoneNozzles 3m	1.45	0.30	1.54	Y	#10, #11, #12, #13, #16, #40, #42
#16 CW Wash 2.5s	3.91	0.48	-1.31	Y	#10, #11, #12, #13, #14, #15, #40, #41, #42
#26 (no Stm) Cool 15s Only	<2.58	1.56	~-0.12	n	
#40 CW HighIntensity 5s	2.35	0.39	-0.14	n	#11, #15, #16
#41 CW HighIntensity 15s	1.90	0.46	0.31	n	#11, #16
#42 CW HighIntensity 30s	2.22	0.32	-0.01	n	#11, #15, #16

**All permutations of final levels are not significantly different except pairings indicated.*

Only treatment #15 showed a significant reduction of 1.54 $\log_{10}$ cfu g^{-1} from control levels. Although significant, treatment #16 showed an increase in levels, and all other cold water configurations exhibited negligible effects on Pseudomonads on breast skin.

10.3.6 Pseudomonads on neck skin (see Table 22)

10.3.6.1 ASC

A summary of all ASC treatments for Pseudomonads on neck skin is given in Table 46.

Table 46. Summary of effect of ASC treatments on pseudomonads on neck skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#01 ASC Mist 10s	2.24	0.13	1.13	Y	#36
#02 ASC Mist 15s	2.20	0.51	0.74	Y	#06, #36
#04 ASC Mist 30s	2.33	0.60	0.90	Y	
#05 ASC Mist+Wait+Rinse 15s+30s+30s	2.16	0.24	0.27	n	#36
#06 ASC Mist+Wait+Rinse 15s+5m+5s	2.76	0.30	0.02	n	#02
#36 ASC Mist+Rinse 10s+5s	2.78	0.41	0.64	n	#01, #02, #05
#37 ASC Mist+Wait+Rinse 10s+5m+5s	2.43	0.38	0.99	Y	
#38 ASC Mist+Wait+Rinse 10s+30m+5s	<2.30	0.80	>1.12	Y	
#39 ASC Mist+Wait+Rinse 10s+60m+5s	2.38	0.51	1.04	Y	

**All permutations of final levels are not significantly different except pairings indicated.*

10.3.6.1.1 Application Time

Treatments #01, #02 and #04 for 10, 15 and 30 seconds misting spray with no rinsing gave significant reductions of 1.13, 0.74 and 0.90 $\log_{10}$ cfu g^{-1}, respectively. No trend was apparent with increasing treatment duration.

10.3.6.1.2 Effects of Rinsing

Treatment #05 was a repeat of the treatment #02 (15 seconds mist) with 30 seconds chemical action time before a 30 seconds rinse designed to give good wash off performance. A decreased reduction from 0.90 to 0.27 $\log_{10}$ cfu g^{-1} was observed. A 15 seconds ASC treatment followed by rinsing gave a result not significantly different from the control.

Treatment #06 with a longer action time and shorter rinse gave higher final levels, and reduced the reduction to negligible levels (0.02 $\log_{10}$ cfu g^{-1}). Treatment #06 did not produce a significant change from untreated control levels.

10.3.6.1.3 Chemical Action (Wait) Time

Treatments #36 to #39 investigated the effect of increasing the chemical action wait time. All wait durations gave significant reductions from control levels; however, there were no obvious trends with increasing chemical action dwell time.

10.3.6.2 TSP

A summary of all TSP treatments for Pseudomonads on neck skin is given in **Error! Reference source not found.**.

Table 47. Summary of effect of TSP treatments on pseudomonads on neck skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#31 TSP Mist 15s	<1.92	1.06	>0.93	n	#32
#32 TSP Mist 30s	<0.81	0.56	>2.01	Y	#31, #33
#33 TSP Mist+Rinse 15s+30s	1.59	0.34	0.76	Y	#32
#34 TSP Mist+Rinse 30s+30s	1.53	0.56	0.82	n	

**All permutations of final levels are not significantly different except pairings indicated.*

10.3.6.2.1 Effects of Application Time

Treatments #31 and #32 for 15 and 30 seconds misting spray with no rinsing gave good reductions of >0.93, and >2.01 $\log_{10}$ cfu g^{-1} respectively. The two treatments showed a trend for increasing reduction and decreasing final levels with longer spraying times, however the treatments were not significantly different from each other. Only treatment #32 (30 seconds) gave a significant change from control levels.

10.3.6.2.2 Effects of Rinsing

Rinse treatments #33 and #34 were not significantly different from each other, yet #33 (15 seconds spray plus 30 seconds rinse) gave significant reduction from control levels. When compared to the non-rinsed treatments #31 and #32 it is seen that rinsing reduces the effect of TSP. The trend for increased reductions and lower final levels with increased spray time as seen for non-rinsed treatments was preserved on rinsed results but to a lesser extent.

10.3.6.3 Steam

A summary of all steam treatments for Pseudomonads on neck skin is given in **Error! Reference source not found.**.

Table 48. Summary of the effect of Steam treatments on pseudomonads on neck skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#26 (no Stm) Cool 15s Only	2.98	1.25	-0.32	n	
#27 Stm Stm 10s	2.33	0.48	0.37	n	#30
#28 Stm Stm 15s	2.24	0.83	0.42	n	#29, #30
#29 Stm Stm+Cool 15s+15s	3.27	0.88	-0.61	n	#28
#30 Stm-Dry Stm+Cool 15s+15s	3.31	0.61	-0.34	n	#27, #28

**All permutations of final levels are not significantly different except pairings indicated.*

10.3.6.3.1 Application Time

Increasing the steam treatment from 10 seconds (#27) to 15 seconds (#28) slightly decreased final levels and improved reductions. However, no stream treatment showed a significant reduction of Pseudomonads on the neck skin between control and treated samples.

10.3.6.3.2 Effects of Cooling Spray

Adding a 15 seconds cooling spray after the 15 seconds steam treatment (#29) was no better than steam alone (#28).

10.3.6.4 Cold Water Spray Configuration

A summary of all cold water treatments with various spray configurations for Pseudomonads on neck skin is given in **Error! Reference source not found.**.

Table 49. Summary of Cold Water Spray Configurations of Pseudomonads on neck skin

Treatment	Mean final level after treatment ($\log_{10}$ cfu g^{-1})	SD	Reduction ($\log_{10}$ cfu g^{-1})	Significant effect of treatment (P<0.01)	Significantly different final levels* (P<0.01)
#10 CW Mist 15s	2.92	0.54	-0.01	n	#11, #15, #16
#11 CW Mist 30s	3.35	0.79	-0.13	n	#10, #13, #14, #15, #16, #41, #42
#12 CW Mist+Wait+Rinse 15s+5m+5s	2.87	0.35	-0.09	n	#16
#13 CW OzoneNozzles 15s	2.73	0.24	0.03	n	#11, #16
#14 CW OzoneNozzles 30s	2.79	0.27	-0.33	n	#11, #16
#15 CW OzoneNozzles 3m	2.25	0.36	1.13	Y	#10, #11, #16
#16 CW Wash 2.5s	4.09	0.24	-1.04	Y	#10, #11, #12, #13, #14, #15, #26, #41, #42
#26 (no Stm) Cool 15s Only	2.98	1.25	-0.32	n	#16
#40 CW HighIntensity 5s	2.97	0.90	-0.58	n	
#41 CW HighIntensity 15s	2.58	0.42	-0.19	n	#11, #16
#42 CW HighIntensity 30s	2.50	0.37	-0.11	n	#11, #16

**All permutations of final levels are not significantly different except pairings indicated.*

Only treatment #15 showed a significant reduction of 1.13 $\log_{10}$ cfu g^{-1} from control levels. Although significant, treatment #16 showed an increase in levels, and all other cold water configurations had negligible effects on Pseudomonads on the neck skin.

10.4 Trends within Results

10.4.1 Summary of treatment duration effects

Summaries of the effects of treatments that were evaluated for two or more durations are given in **Error! Reference source not found.** to **Error! Reference source not found.**. Each table shows the summary for a particular organism-type/skin-part combination, and assessed for benefit with mean comparison data of: a). mean $\log_{10}$ microbial levels before treatment, b). mean $\log_{10}$ reductions achieved by the treatment compared to water alone; c). proportion of samples after treatment below the limit of detection (LoD) and d). the numbers of microbes remaining after treatment.

Table 50. Summary of increasing treatment duration for campylobacters on breast skin

Treatment	Criteria	Treatment Duration					Benefit with increasing duration*
		5s	10s	15s	30s	3m	
ASC Mist	n		5	20	15		-
	log_{10} Cntrl (SD)		2.00 (0.87)	<1.52 (0.67)	1.73 (0.62)		-
	log_{10} Treatd (SD)		<0.43(0.26)	<0.67 (0.61)	<0.45 (0.17)		V
	log_{10} Reductn		**>1.57**	~0.85	>1.28		?
	%<LoD incrse		100%	70%	93%		V
CW Mist	n			30	55		-
	log_{10} Cntrl (SD)			<2.28 (0.90)	<2.12 (0.79)		-
	log_{10} Treatd (SD)			<2.25 (0.73)	1.93 (0.52)		Y
	log_{10} Reductn			~0.03	<0.19		?
	%<LoD incrse			0%	-2%		N
CW Deluge	n			5	5	5	-
	log_{10} Cntrl (SD)			2.28 (0.14)	2.28 (0.14)	1.72 (0.51)	-
	log_{10} Treatd (SD)			1.60 (0.33)	2.02 (0.33)	2.02 (0.13)	N
	log_{10} Reductn			0.68	0.26	-0.30	Y
	%<LoD incrse			0%	0%	0%	?
PAA Mist	n			10	10		-
	log_{10} Cntrl (SD)			2.93 (0.94)	2.93 (0.94)		-
	log_{10} Treatd (SD)			2.12 (0.83)	<1.78 (0.75)		Y
	log_{10} Reductn			0.81	>1.15		Y
	%<LoD incrse			0%	20%		Y
Steam	n		5	15			-
	log_{10} Cntrl (SD)		1.31 (0.42)	<2.15 (0.83)			-
	log_{10} Treatd (SD)		1.50 (0.45)	<0.87 (0.63)			Y
	log_{10} Reductn		-0.19	~1.28			Y
	%<LoD incrse		0%	47%			Y
TSP Mist	n			10	15		-
	log_{10} Cntrl (SD)			2.50 (0.21)	<2.16 (0.57)		-
	log_{10} Treatd (SD)			1.92 (0.61)	<0.79 (0.38)		Y
	log_{10} Reductn			0.58	~1.37		Y
	%<LoD incrse			0%	60%		Y
CW High Intensity Wash	n	5		5	5		-
	log_{10} Cntrl (SD)	2.71 (0.39)		2.71 (0.39)	2.71 (0.39)		-
	log_{10} Treatd (SD)	2.13 (0.48)		1.98 (1.09)	2.12 (0.15)		V
	log_{10} Reductn	0.58		0.73	0.59		V
	%<LoD incrse	0%		0%	0%		?

*KEY:

Y = Yes (improvement with every increase in treatment duration)

V = Variable (both improvements and declines with increasing duration)

? = Indeterminate (no change in values or inequalities)

N = No (worsening with every increase in treatment duration)

For Campylobacters on breast skin it can be seen that generally increasing treatment duration does improve performance for most assessment methods.

Table 51. Summary of increasing treatment duration for Campylobacters on neck skin

Treatment	Criteria	Treatment Duration					Benefit with increasing duration*
		5s	10s	15s	30s	3m	
ASC Mist	n		5	20	15		-
	log_{10} Cntrl (SD)		2.86 (1.37)	2.41 (0.87)	2.45 (0.91)		-
	log_{10} Treatd (SD)		<1.41 (1.47)	<0.96 (0.92)	<0.85 (0.60)		Y
	log_{10} Reductn		>1.45	**>1.45**	**>1.60**		Y
	%<LoD incrse		20%	65%	47%		V
CW Mist	n			30	55		-
	log_{10} Cntrl (SD)			3.01 (0.85)	2.81 (0.85)		-
	log_{10} Treatd (SD)			2.78 (0.84)	2.41 (0.61)		Y
	log_{10} Reductn			0.23	**0.40**		Y
	%<LoD incrse			0%	0%		?
CW Deluge	n			5	5	5	-
	log_{10} Cntrl (SD)			2.98 (0.46)	2.98 (0.46)	1.98 (0.14)	-
	log_{10} Treatd (SD)			2.43 (0.04)	2.91 (0.60)	3.02 (0.45)	N
	log_{10} Reductn			0.55	0.07	-1.04	N
	%<LoD incrse			0%	0%	0%	?
PAA Mist	n			10	10		-
	log_{10} Cntrl (SD)			3.58 (0.84)	3.58 (0.84)		-
	log_{10} Treatd (SD)			2.62 (0.73)	2.61 (0.89)		Y
	log_{10} Reductn			0.96	0.97		Y
	%<LoD incrse			0%	0%		?
Steam	n		5	15			-
	log_{10} Cntrl (SD)		1.57 (0.40)	2.58 (1.03)			-
	log_{10} Treatd (SD)		2.35 (0.22)	2.05 (0.85)			Y
	log_{10} Reductn		-0.78	0.53			Y
	%<LoD incrse		0%	0%			?
TSP Mist	n			10	15		-
	log_{10} Cntrl (SD)			3.25 (0.28)	3.22 (0.46)		-
	log_{10} Treatd (SD)			<1.89 (0.1.22)	<0.81 (0.74)		Y
	log_{10} Reductn			>1.36	**>2.41**		Y
	%<LoD incrse			20%	73%		Y
CW High Intensity Wash	n	5		5	5		-
	log_{10} Cntrl (SD)	3.05 (0.40)		3.05 (0.40)	3.05 (0.40)		-
	log_{10} Treatd (SD)	2.91 (0.49)		2.68 (0.40)	2.93 (0.49)		V
	log_{10} Reductn	0.14		0.37	0.12		V
	%<LoD incrse	0%		0%	0%		?

*KEY:

Y = Yes (improvement with every increase in treatment duration)

V = Variable (both improvements and declines with increasing duration)

? = Indeterminate (no change in values or inequalities)

N = No (worsening with every increase in treatment duration)

Predominantly for Campylobacters on neck skin it can be seen that increasing treatment duration does generally improve performance for most assessment methods. It is interesting to note that the deluge treatments appeared to have a consistent contaminating effect with increased duration for both assessment methods that showed a difference.

Table 52. Summary of increasing treatment duration for Enterobacteriaceae on breast skin

Treatment	Criteria	Treatment Duration					Benefit with increasing duration*
		5s	10s	15s	30s	3m	
ASC Mist	n		5	20	15		-
	log_{10} Cntrl (SD)		3.01 (0.76)	2.89 (0.54)	3.27 (0.52)		-
	log_{10} Treatd (SD)		<0.61 (0.32)	<1.31 (0.87)	<1.17 (0.87)		V
	log_{10} Reductn		**>2.40**	**>1.58**	**>2.10**		V
	%<LoD incrse		40%	45%	47%		Y
CW Mist	n			30	55		-
	log_{10} Cntrl (SD)			3.30 (0.75)	3.79 (1.06)		-
	log_{10} Treatd (SD)			3.53 (0.73)	3.66 (0.88)		N
	log_{10} Reductn			-0.23	0.13		Y
	%<LoD incrse			0%	0%		?
CW Deluge	n			5	5	5	-
	log_{10} Cntrl (SD)			2.86 (0.46)	2.86 (0.46)	3.06 (0.60)	-
	log_{10} Treatd (SD)			3.33 (0.21)	3.02 (0.37)	2.66 (0.52)	Y
	log_{10} Reductn			>0.47	>0.16	0.40	N
	%<LoD incrse			0%	0%	0%	?
PAA Mist	n			10	10		-
	log_{10} Cntrl (SD)			3.36 (0.83)	3.36 (0.83)		-
	log_{10} Treatd (SD)			3.17 (0.80)	2.76 (0.53)		Y
	log_{10} Reductn			0.19	0.60		Y
	%<LoD incrse			0%	0%		?
Steam	n		5	15			-
	log_{10} Cntrl (SD)		3.18 (0.39)	3.28 (0.36)			-
	log_{10} Treatd (SD)		2.88 (0.31)	<2.26 (0.99)			Y
	log_{10} Reductn		0.30	**>1.02**			Y
	%<LoD incrse		0%	7%			Y
TSP Mist	n			10	15		-
	log_{10} Cntrl (SD)			3.67 (0.68)	3.14 (0.99)		-
	log_{10} Treatd (SD)			3.49 (0.89)	<2.13 (1.26)		Y
	log_{10} Reductn			0.89	>1.26		Y
	%<LoD incrse			0%	27%		Y
CW High Intensity Wash	n	5		5	5		-
	log_{10} Cntrl (SD)	3.26 (0.19)		3.26 (0.19)	3.26 (0.19)		-
	log_{10} Treatd (SD)	3.19 (0.17)		3.43 (0.49)	3.46 (0.25)		N
	log_{10} Reductn	0.07		-0.17	-0.20		N
	%<LoD incrse	0%		0%	0%		?

*KEY:

Y = Yes (improvement with every increase in treatment duration)

V = Variable (both improvements and declines with increasing duration)

? = Indeterminate (no change in values or inequalities)

N = No (worsening with every increase in treatment duration)

For Enterobacteriaceae on breast skin only steam and TSP treatment showed consistent improvements in performance with increasing treatment duration.

Table 53. Summary of increasing treatment duration for Enterobacteriaceae on neck skin

Treatment	Criteria	Treatment Duration					Benefit with increasing duration*
		5s	10s	15s	30s	3m	
ASC Mist	n		5	20	15		-
	log_{10} Cntrl (SD)		3.59 (0.83)	3.58 (0.67)	3.96 (0.64)		-
	log_{10} Treatd (SD)		<1.41 (0.87)	<1.81 (1.00)	<2.07 (0.80)		N
	log_{10} Reductn		**>2.18**	**>1.77**	**>1.89**		V
	%<LoD incrse		20%	15%	7%		N
CW Mist	n			30	55		-
	log_{10} Cntrl (SD)			4.01 (0.83)	4.16 (0.94)		-
	log_{10} Treatd (SD)			3.94 (0.58)	3.78 (0.79)		Y
	log_{10} Reductn			0.07	0.38		Y
	%<LoD incrse			0%	0%		?
CW Deluge	n			5	5	5	-
	log_{10} Cntrl (SD)			3.55 (0.33)	3.55 (0.33)	3.36 (0.34)	-
	log_{10} Treatd (SD)			3.69 (0.32)	3.72 (0.40)	3.41 (0.31)	V
	log_{10} Reductn			-0.14	-0.17	-0.05	V
	%<LoD incrse			0%	0%	0%	?
PAA Mist	n			10	10		-
	log_{10} Cntrl (SD)			3.97 (0.69)	3.97 (0.69)		-
	log_{10} Treatd (SD)			3.17 (0.64)	3.21 (0.46)		N
	log_{10} Reductn			0.80	0.76		N
	%<LoD incrse			0%	0%		?
Steam	n		5	15			-
	log_{10} Cntrl (SD)		3.70 (0.43)	3.72 (0.48)			-
	log_{10} Treatd (SD)		3.71 (0.74)	3.70 (0.57)			Y
	log_{10} Reductn		-0.01	0.02			Y
	%<LoD incrse		0%	0%			?
TSP Mist	n			10	15		-
	log_{10} Cntrl (SD)			4.29 (1.04)	4.17 (0.90)		-
	log_{10} Treatd (SD)			2.59 (1.62)	<0.88 (0.83)		Y
	log_{10} Reductn			1.70	**>3.29**		Y
	%<LoD incrse			0%	67%		Y
CW High Intensity Wash	n	5		5	5		-
	log_{10} Cntrl (SD)	3.65 (0.37)		3.65 (0.37)	3.65 (0.37)		-
	log_{10} Treatd (SD)	3.79 (0.60)		3.64 (0.46)	3.85 (0.24)		V
	log_{10} Reductn	-0.14		0.01	-0.20		V
	%<LoD incrse	0%		0%	0%		?

*KEY:

Y = Yes (improvement with every increase in treatment duration)

V = Variable (both improvements and declines with increasing duration)

? = Indeterminate (no change in values or inequalities)

N = No (worsening with every increase in treatment duration)

For Enterobacteriaceae on neck skin it can be seen that increasing treatment duration does not necessarily improve performance. For PAA misting there appears to be a small contaminating effect in these data of increasing treatment duration.

Table 54. Summary of increasing treatment duration for Pseudomonads on breast skin

Treatment	Criteria	Treatment Duration					Benefit with increasing duration*
		5s	10s	15s	30s	3m	
ASC Mist	n		5	20	15		-
	log_{10} Cntrl (SD)		3.29 (0.12)	2.49 (0.65)	2.78 (0.48)		-
	log_{10} Treatd (SD)		<1.29 (0.78	<1.33 (0.70)	<1.57 (0.71)		N
	log_{10} Reductn		**>2.00**	**>1.16**	**>1.21**		V
	%<LoD incrse		40%	25%	20%		N
CW Mist	n			30	55		-
	log_{10} Cntrl (SD)			2.29 (0.63)	<2.78 (0.90)		-
	log_{10} Treatd (SD)			2.42 (0.64)	3.07 (0.95)		N
	log_{10} Reductn			-0.13	<-0.29		N
	%<LoD incrse			0%	-4%		N
CW Deluge	n			5	5	5	-
	log_{10} Cntrl (SD)			2.05 (0.42)	2.05 (0.42)	2.99 (0.51)	-
	log_{10} Treatd (SD)			2.38 (0.27)	2.11 (0.26)	1.45 (0.30)	Y
	log_{10} Reductn			-0.33	-0.06	**1.54**	Y
	%<LoD incrse			0%	0%	0%	?
PAA Mist	n			10	10		-
	log_{10} Cntrl (SD)			2.37 (0.67)	2.37 (0.67)		-
	log_{10} Treatd (SD)			2.00 (0.52)	1.81 (0.52)		Y
	log_{10} Reductn			0.37	0.56		Y
	%<LoD incrse			0%	0%		?
Steam	n		5	15			-
	log_{10} Cntrl (SD)		2.39 (0.36)	2.46 (0.44)			-
	log_{10} Treatd (SD)		1.33 (0.34)	<1.44 (0.77)			N
	log_{10} Reductn		**1.06**	**>1.32**			Y
	%<LoD incrse		0%	40%			Y
TSP Mist	n			10	15		-
	log_{10} Cntrl (SD)			2.45 (0.68)	<1.98 (0.90)		-
	log_{10} Treatd (SD)			<2.02 (0.90)	<1.23 (0.76)		Y
	log_{10} Reductn			>0.43	~0.75		?
	%<LoD incrse			10%	27%		Y
CW High Intensity Wash	n	5		5	5		-
	log_{10} Cntrl (SD)	2.21 (0.12)		2.21 (0.12)	2.21 (0.12)		-
	log_{10} Treatd (SD)	2.35 (0.39)		1.90 (0.46)	2.22 (0.32)		V
	log_{10} Reductn	-0.14		0.31	-0.01		V
	%<LoD incrse	0%		0%	0%		?

*KEY:

Y = Yes (improvement with every increase in treatment duration)

V = Variable (both improvements and declines with increasing duration)

? = Indeterminate (no change in values or inequalities)

N = No (worsening with every increase in treatment duration)

For Pseudomonads on breast skin it can be seen that increasing treatment duration does not necessarily improve performance. For cold water misting there appears to be a contaminating effect of increasing treatment duration.

Table 55. Summary of increasing treatment duration for Pseudomonads on neck skin

Treatment	Criteria	Treatment Duration					Benefit with increasing duration*
		5s	10s	15s	30s	3m	
ASC Mist	n		5	20	15		-
	log_{10} Cntrl (SD)		3.37 (0.24)	2.94 (0.47)	3.23 (0.37)		-
	log_{10} Treatd (SD)		2.24 (0.13)	2.20 (0.51)	2.33 (0.60)		V
	log_{10} Reductn		**1.13**	**0.74**	**0.90**		V
	%<LoD incrse		0%	0%	0%		?
CW Mist	n			30	55		-
	log_{10} Cntrl (SD)			2.91 (0.56)	3.22 (0.77)		-
	log_{10} Treatd (SD)			2.92 (0.54)	3.35 (0.79)		N
	log_{10} Reductn			-0.01	-0.13		N
	%<LoD incrse			0%	0%		?
CW Deluge	n			5	5	5	-
	log_{10} Cntrl (SD)			2.76 (0.28)	2.46 (0.28)	3.38 (0.08)	-
	log_{10} Treatd (SD)			2.73 (0.24)	2.79 (0.27)	2.25 (0.36)	V
	log_{10} Reductn			0.03	-0.33	**1.13**	Y
	%<LoD incrse			0%	0%	0%	?
PAA Mist	n			10	10		-
	log_{10} Cntrl (SD)			3.08 (0.53)	3.08 (0.53)		-
	log_{10} Treatd (SD)			2.29 (0.30)	2.33 (0.22)		N
	log_{10} Reductn			**0.79**	**0.75**		N
	%<LoD incrse			0%	0%		?
Steam	n		5	15			-
	log_{10} Cntrl (SD)		2.70 (0.43)	2.66 (0.53)			-
	log_{10} Treatd (SD)		2.33 (0.48)	2.24 (0.83)			Y
	log_{10} Reductn		0.37	0.42			Y
	%<LoD incrse		0%	0%			?
TSP Mist	n			10	15		-
	log_{10} Cntrl (SD)			2.85 (0.62)	2.82 (0.51)		-
	log_{10} Treatd (SD)			<1.92 (1.06)	<0.81 (0.56)		Y
	log_{10} Reductn			>0.93	**>2.01**		Y
	%<LoD incrse			10%	53%		Y
CW High Intensity Wash	n	5		5	5		-
	log_{10} Cntrl (SD)	2.39 (0.24)		2.39 (0.24)	2.39 (0.24)		-
	log_{10} Treatd (SD)	2.97 (0.90)		2.58 (0.42)	2.50 (0.37)		Y
	log_{10} Reductn	-0.58		-0.19	-0.11		Y
	%<LoD incrse	0%		0%	0%		?

*KEY:

Y = Yes (improvement with every increase in treatment duration)

V = Variable (both improvements and declines with increasing duration)

? = Indeterminate (no change in values or inequalities)

N = No (worsening with every increase in treatment duration)

For Pseudomonads on neck skin it can be seen that increasing treatment duration does not necessarily improve performance. For cold water and PAA misting there appears to be a contaminating effect of increasing treatment duration.

10.4.1.1 Effect of treatment duration - conclusions

Overall, increasing treatment duration does not necessarily improve performance. Additionally, it should be borne in mind that these chemical treatments are unrinsed (rinsing would be required under EFSA guidelines) and rinsing has a substantial detrimental effect on the performance of chemical treatments (**Error! Reference source not found.** to **Error! Reference source not found.**).

For some cold water only treatments (deluge for Campylobacters on neck skin, high-intensity for Enterobacteriaceae on breast skin, mist for Pseudomonads on breast skin and neck skin), there appears to be a contaminating effect with increasing duration for assessment methods that showed a difference.

Due to the greater number of duration datasets, treatments with three instances of different durations showed clear benefits less often than treatments with only two duration instances.

10.4.2 Summary of rinsing effects.

The effects of rinsing after chemical treatment were assessed for the two best performing chemicals (ASC and TSP). Samples treated with ASC for 10 seconds were rinsed for 5 seconds, and samples treated with TSP for 15 and 30 seconds were rinsed for 30 seconds. These data were then compared to unrinsed treatments of the same duration (**Error! Reference source not found.** to **Error! Reference source not found.**).

Table 56 . Effects of rinsing after 10 seconds ASC mist treatment

Organism / Carcass part	Criteria	No rinse [§01] (n=5)	5s Rinse [§36] (n=10)	Benefit with rinse*
Campy	$\log_{10}$ Cntrl (SD)	2.00 (0.87)	2.65 (0.36)	-
Breast skin	$\log_{10}$ Treatd (SD)	<0.43(0.26)	<1.39 (0.79)	N
	$\log_{10}$ Reductn	**>1.57**	**>1.26**	N
	%<LoD incrse	100%	22%	N
Campy	$\log_{10}$ Cntrl (SD)	2.86 (1.37)	2.88 (0.44)	-
Neck skin	$\log_{10}$ Treatd (SD)	<1.41 (1.47)	<1.80 (0.73)	N
	$\log_{10}$ Reductn	>1.45	**>1.08**	N
	%<LoD incrse	20%	11%	N
Entero	$\log_{10}$ Cntrl (SD)	3.01 (0.76)	3.70 (0.53)	-
Breast skin	$\log_{10}$ Treatd (SD)	<0.61 (0.32)	3.14 (0.62)	N
	$\log_{10}$ Reductn	**>2.40**	0.56	N
	%<LoD incrse	40%	0%	N
Entero	$\log_{10}$ Cntrl (SD)	3.59 (0.83)	4.01 (0.57)	-
Neck skin	$\log_{10}$ Treatd (SD)	<1.41 (0.87)	3.30 (0.45)	N
	$\log_{10}$ Reductn	**>2.18**	0.71	N
	%<LoD incrse	20%	0%	N
Pseudo	$\log_{10}$ Cntrl (SD)	3.29 (0.12)	2.99 (0.58)	-
Breast skin	$\log_{10}$ Treatd (SD)	<1.29 (0.78	2.70 (0.46)	N
	$\log_{10}$ Reductn	**>2.00**	0.29	N
	%<LoD incrse	40%	0%	N
Pseudo	$\log_{10}$ Cntrl (SD)	3.37 (0.24)	3.42 (0.69)	-
Neck skin	$\log_{10}$ Treatd (SD)	2.24 (0.13)	2.78 (0.41)	N
	$\log_{10}$ Reductn	**1.13**	-0.64	N
	%<LoD incrse	0%	0%	?

Table 57. Effects of rinsing after 15 seconds TSP mist treatment

Organism / Carcass part	Criteria	No rinse [§31] (n=10)	30 s Rinse [§33] (n=5)	Benefit with rinse*
Campy	$\log_{10}$ Cntrl (SD)	2.50 (0.21)	2.37 (0.16)	-
Breastskin	$\log_{10}$ Treatd (SD)	1.92 (0.61)	1.79 (0.35)	N
	$\log_{10}$ Reductn	0.58	0.58	?
	%<LoD incrse	0%	0%	?
Campy	$\log_{10}$ Cntrl (SD)	3.25 (0.28)	3.24 (0.18)	-
Neck skin	$\log_{10}$ Treatd (SD)	<1.89 (0.1.22)	2.25 (0.42)	N
	$\log_{10}$ Reductn	>1.36	**0.99**	N
	%<LoD incrse	20%	0%	N
Entero	$\log_{10}$ Cntrl (SD)	3.67 (0.68)	3.07 (0.19)	-
Breastskin	$\log_{10}$ Treatd (SD)	3.49 (0.89)	2.73 (0.27	Y
	$\log_{10}$ Reductn	0.89	0.27	N
	%<LoD incrse	0%	0%	?
Entero	$\log_{10}$ Cntrl (SD)	4.29 (1.04)	3.34 (0.21)	-
Neck skin	$\log_{10}$ Treatd (SD)	2.59 (1.62)	2.86 (0.48)	N
	$\log_{10}$ Reductn	1.70	0.48	N
	%<LoD incrse	0%	0%	?
Pseudo	$\log_{10}$ Cntrl (SD)	2.45 (0.68)	1.83 (0.23)	-
Breastskin	$\log_{10}$ Treatd (SD)	<2.02 (0.90)	1.42 (0.42)	Y
	$\log_{10}$ Reductn	>0.43	0.41	N
	%<LoD incrse	10%	0%	N
Pseudo	$\log_{10}$ Cntrl (SD)	2.85 (0.62)	2.35 (0.24)	-
Neck skin	$\log_{10}$ Treatd (SD)	<1.92 (1.06)	1.59 (0.34)	Y
	$\log_{10}$ Reductn	>0.93	0.76	N
	%<LoD incrse	10%	0%	N

Table 58. Effects of rinsing after 30 seconds TSP mist treatment

Organism / Carcass part	Criteria	No rinse [§32] (n=15)	30 s Rinse [§34] (n=5)	Benefit with rinse*
Campy	log_{10} Cntrl (SD)	<2.16 (0.57)	2.37 (0.16)	-
Breastskin	log_{10} Treatd (SD)	<0.79 (0.38)	1.50 (0.28)	N
	log_{10} Reductn	**~1.37**	**0.87**	N
	%<LoD incrse	60%	0%	N
Campy	log_{10} Cntrl (SD)	3.22 (0.46)	3.24 (0.18)	-
Neck skin	log_{10} Treatd (SD)	<0.81 (0.74)	2.11 (0.52)	N
	log_{10} Reductn	**>2.41**	**1.13**	N
	%<LoD incrse	73%	0%	N
Entero	log_{10} Cntrl (SD)	3.14 (0.99)	3.07 (0.19)	-
Breastskin	log_{10} Treatd (SD)	<2.13 (1.26)	2.80 (0.27	N
	log_{10} Reductn	>1.26	0.27	N
	%<LoD incrse	27%	0%	N
Entero	log_{10} Cntrl (SD)	4.17 (0.90)	3.34 (0.21)	-
Neck skin	log_{10} Treatd (SD)	<0.88 (0.83)	2.89 (0.22)	N
	log_{10} Reductn	**>3.29**	0.45	N
	%<LoD incrse	67%	0%	N
Pseudo	log_{10} Cntrl (SD)	<1.98 (0.90)	1.83 (0.23)	-
Breastskin	log_{10} Treatd (SD)	<1.23 (0.76)	1.37 (0.61)	N
	log_{10} Reductn	~0.75	0.46	N
	%<LoD incrse	27%	0%	N
Pseudo	log_{10} Cntrl (SD)	2.82 (0.51)	2.35 (0.24)	-
Neck skin	log_{10} Treatd (SD)	<0.81 (0.56)	1.53 (0.56)	N
	log_{10} Reductn	**>2.01**	0.82	N
	%<LoD incrse	53%	0%	N

10.4.2.1 Effect of rinsing - conclusions

Adding a 5 second rinse after a 10 second ASC treatment or a 30 second rinse after 30 seconds TSP treatment reduced efficacy of overall treatment for all organism-type/bird-part combinations by all assessment methods. Adding a 30 seconds rinse after a 15 seconds TSP treatment reduced the overall efficacy for most organism-type/bird-part combinations under most assessment methods. However, final treated levels after rinse for Enterobacteriaceae on the breast skin, and Pseudomonads on breast and neck skin showed a small benefit of rinsing.

Predominantly, rinsing substantially reduces efficacy of chemical decontaminants.

10.4.3 Summary of chemical action time (dwell) effects.

The effects of varying the chemical action time (dwell) were assessed for the best performing chemical (ASC). Samples were treated with ASC for 10 seconds and then rinsed for 5 seconds after delays of 0, 5, 30 and 60 minutes (**Error! Reference source not found.**, **Error! Reference source not found.**).

Table 59. Effects of dwell time before 5 second rinse after 10 seconds ASC treatment

Organism / Carcass part	Criteria	No rinse [§01] (n=5)	Dwell 0m [§36] (n=10)	Dwell 5m [§37] (n=10)	Dwell 30m [§38] (n=10)	Dwell 60m [§39] (n=10)
Campy Breast skin	log_{10} Cntrl (SD)	2.00 (0.87)	2.65 (0.36)	2.65 (0.36)	2.65 (0.36)	2.65 (0.36)
	log_{10} Treatd (SD)	<0.43(0.26)	<1.39 (0.79)	<0.83 (0.62)	<1.38 (0.65)	1.70 (0.63)
	log_{10} Reductn	**>1.57**	**>1.26**	**>1.82**	**>1.27**	**0.95**
	%<LoD incrse	100%	22%	40%	10%	0%
Campy Neck skin	log_{10} Cntrl (SD)	2.86 (1.37)	2.88 (0.44)	2.88 (0.44)	2.88 (0.44)	2.88 (0.44)
	log_{10} Treatd (SD)	<1.41 (1.47)	<1.80 (0.73)	<1.76 (0.61)	2.33 (0.54)	<1.89 (1.07)
	log_{10} Reductn	>1.45	**>1.08**	**>1.12**	0.55	>0.99
	%<LoD incrse	20%	11%	10%	0%	20%
Entero Breast skin	log_{10} Cntrl (SD)	3.01 (0.76)	3.70 (0.53)	3.70 (0.53)	3.70 (0.53)	3.70 (0.53)
	log_{10} Treatd (SD)	<0.61 (0.32)	3.14 (0.62)	3.53 (0.36)	2.92 (0.69)	3.11 (0.59)
	log_{10} Reductn	**>2.40**	0.56	0.17	0.78	0.59
	%<LoD incrse	40%	0%	0%	0%	0%
Entero Neck skin	log_{10} Cntrl (SD)	3.59 (0.83)	4.01 (0.57)	4.01 (0.57)	4.01 (0.57)	4.01 (0.57)
	log_{10} Treatd (SD)	<1.41 (0.87)	3.30 (0.45)	3.80 (0.43)	3.37 (0.69)	3.32 (0.76)
	log_{10} Reductn	**>2.18**	0.71	0.21	0.64	0.69
	%<LoD incrse	20%	0%	0%	0%	0%
Pseudo Breast skin	log_{10} Cntrl (SD)	3.29 (0.12)	2.99 (0.58)	2.99 (0.58)	2.99 (0.58)	2.99 (0.58)
	log_{10} Treatd (SD)	<1.29 (0.78	2.70 (0.46)	2.33 (0.55)	2.00 (0.70)	2.08 (0.75)
	log_{10} Reductn	**>2.00**	0.29	0.66	**0.99**	**0.91**
	%<LoD incrse	40%	0%	0%	0%	0%
Pseudo Neck skin	log_{10} Cntrl (SD)	3.37 (0.24)	3.42 (0.69)	3.42 (0.69)	3.42 (0.69)	3.42 (0.69)
	log_{10} Treatd (SD)	2.24 (0.13)	2.78 (0.41)	2.43 (0.38)	<2.30 (0.80)	2.38 (0.51)
	log_{10} Reductn	**1.13**	-0.64	**0.99**	**>1.12**	**1.04**
	%<LoD incrse	0%	0%	0%	10%	0%

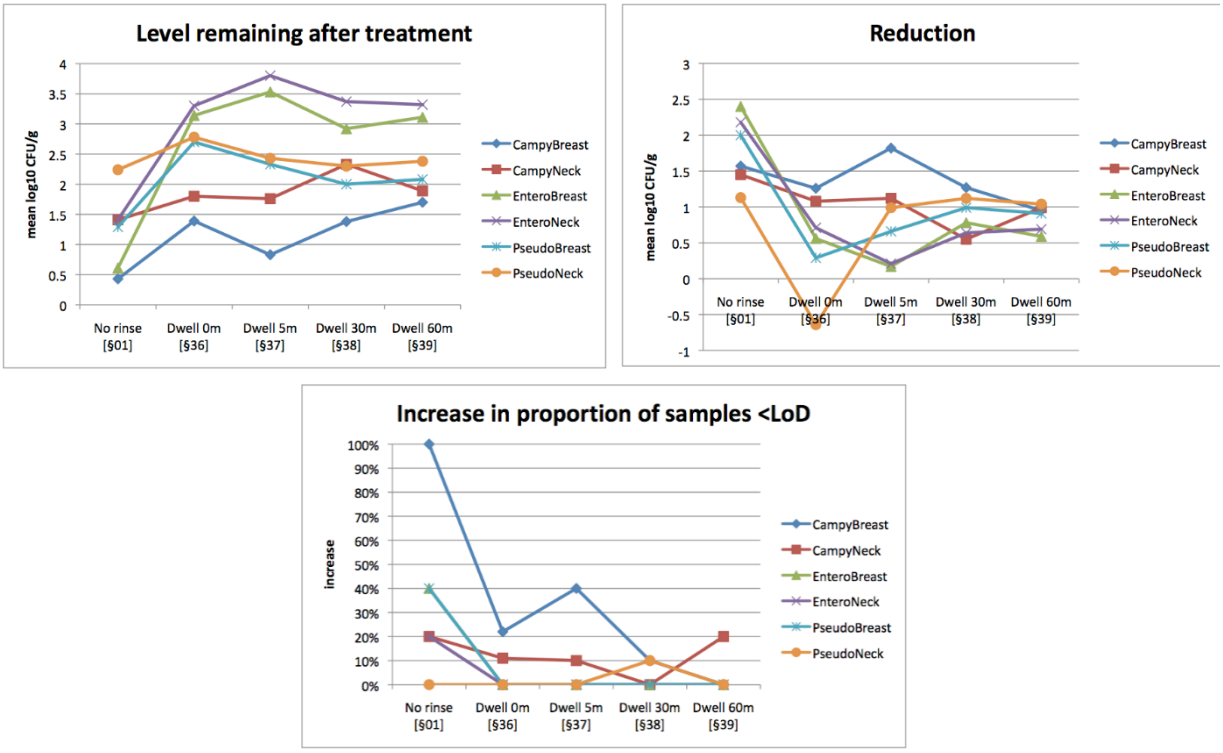


Figure 32. Effects of chemical action time (dwell) before rinse

It can be seen from **Error! Reference source not found.** and **Error! Reference source not found.** that whilst any rinsing substantially reduces the efficacy of the treatment, there is generally a benefit in increasing the dwell time before rinsing.

10.4.4 Conclusions from experimental work

- ASC (unrinsed) is the most effective chemical treatment.

- Steam is the most effective physical treatment.
- Increasing the treatment duration does not necessarily increase anti-microbial efficacy (not withstanding any surface change limitations with increased treatment durations)
- Rinsing substantially reduces the anti-microbial efficacy of chemical treatments.
- Allowing a longer dwell time before rinsing tends to increase the efficacy of rinsed treatments.

11 Investigation to see whether a flail washer reduced Campylobacter contamination of chicken carcasses in Plant number 4

These experiments were carried out on 14th November and 12th December 2006 in plant no. 4 in order to assess whether the flail washers in plant numbers 2 and 4 were effective in reducing carcass contamination with Campylobacter, Enterobacteriaceae and Pseudomonads. The two machines were of the same design.

11.1 Methods

The flail washer was situated immediately before the inside-outside washer. During a meal break, 60 carcasses were retained on the line. Twenty carcasses were sampled immediately before passing through the flail washer. Twenty carcasses were sampled immediately after passing through the flail washer and 20 carcasses were sampled after passing through the flail washer and then the inside-outside washer. Neck skin and breast skin were sampled. Samples were stored chilled, and examined the following day.

Neck flaps Approximately 30 g of skin (avoiding fat) were added to 70 ml MRD and homogenised using the Pulverizer (30 seconds).

Breast skin Two 10 cm^2 samples per carcass, were taken from diametrically opposite ends of the breast, added to 20 ml MRD (to yield one suspension per carcass), and homogenised using the Pulverizer (30 seconds).

Caecal contents were examined from 13 and 17 carcasses on 14th November and 12th December respectively.

Microbiological examination. Decimal dilutions were prepared and plate counts of Campylobacter, Enterobacteriaceae and pseudomonads carried out as described in Section 6.1.1. Results were assessed using ANOVA.

11.2 Results

The results are shown in Table 60. Numbers of all bacterial groups were lower on the breast- than the neck skin on both days. All 13 caecal contents were positive for Campylobacter on 14th November (mean 7.3 $\log_{10}$ cfu g^{-1}), while on 12th December only 1/17 was positive (7.01 $\log_{10}$ cfu g^{-1}).

Results from 14th November. For the Enterobacteriaceae there was a significant difference between the numbers on the breast skin ($p<0.001$), with the counts after the flail washer and after the inside/outside washer being significantly lower than those before the flail washer. For the Pseudomonads, no significant effect was observed. Numbers of Campylobacters were low and many results were below the limit of detection (10 cfu g^{-1}). Mean numbers on the positive samples were not significantly lower after the flail washer.

Results from 12th December. For both neck and breast skin there was a significantly lower count of Enterobacteriaceae after the inside/outside washer compared to other points in the sequence. For Pseudomonads, there was a significantly lower count on the neck skin after the flail washer compared to other points in the sequence. On the breast skin, there were overlapping significancies between Pseudomonad counts at the three positions, but counts were significantly lower after the flail washer than before it. Numbers of Campylobacters were low and many results were below the limit of detection (10 cfu g^{-1}). Mean numbers on the positive samples were not significantly lower after the flail washer.

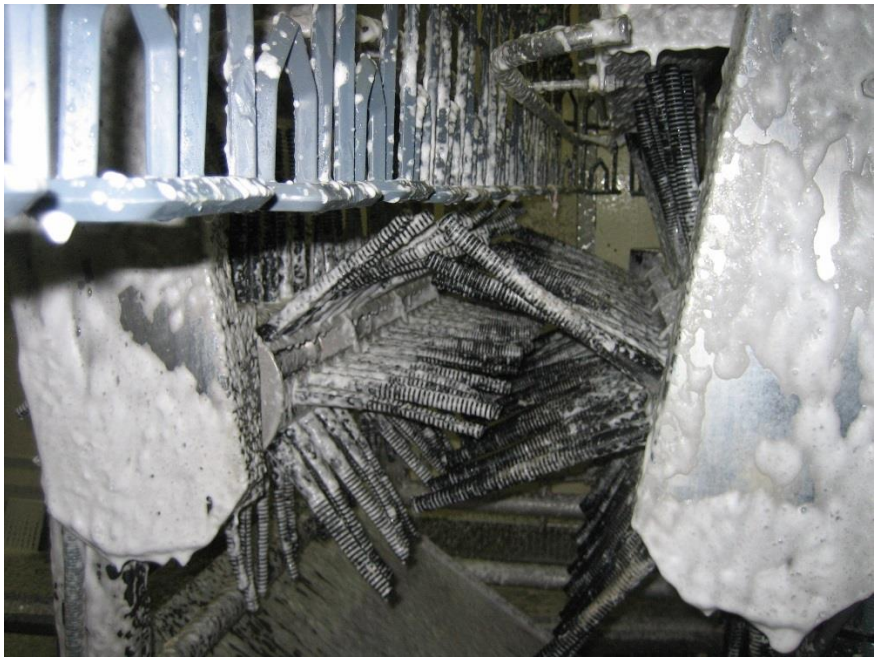


Figure 33. Flail washer during cleaning, Plant number 4

11.3 Discussion and Conclusions

A clear effect of the flail washer in reducing numbers of any of the three groups of bacteria on chicken carcasses was not observed. Although similar numbers of Campylobacters were detected on the carcasses on the two occasions, numbers in the caeca were much lower on 12th December. It is sometimes difficult to be sure that carcasses and caeca both originate from the same flock – and on 12th December this might not have been the case. It was clear that numbers of all bacterial groups were lower on breast- than neck skin.

Further work was not carried out on flail washers because of the lack of a clear beneficial effect, and also because the plants that used them were both closed within the next few years. Also, the machines were both built by the company that constructed the plants, and not available commercially.

Table 60. Summary of results from flail washer (mean log_{10} cfu g^{-1} or cm^2 n=20)

14th November 2006

	Enterobacteriaceae	Pseudomonads	Campylobacter (no. +ve/20)	Campylobacter mean of +ves
Neck				
Before flail	5.18	5.10	10/20	2.48
After flail	4.73	5.17	12/20	2.52
After I/O washer	4.94	5.33	4/19	2.56
Breast				
Before flail	3.04	2.59	12/20	1.78
After flail	1.83*	2.34	1/20	2.1
After I/O washer	2.21*	2.42	1/19	1.81

12th December 2006

	Enterobacteriaceae	Pseudomonads	Campylobacter (no.+ve/20)	Campylobacter mean of +ves
Neck				
Before flail	6.58	5.88	11/20	2.72
After flail	5.93	5.42*	13/20	2.68
After I/O washer	5.37*	5.89*	12/20	2.22
Breast				
Before flail	2.11	3.28	9/20	1.73
After flail	1.91	2.59*	6/20	1.71
After I/O washer	1.50*	2.90	6/20	1.59

* significant difference ($p = <0.05$) from previous count

12 Recommendations for a best practice guide on-line

The research team collaborated with the team, led by Dr Michael Hutchison, working on FSA Project number MO1045 "Evaluation of data generated by meat plants for current HACCP verification purposes and future slaughterhouse hygiene purposes" (FSA MO1045 Report, 2008). Part of the work carried out in this project identified hazards to product integrity/hygiene for each stage of processing for each of the four meat species (cattle, sheep, pigs and poultry) processed in the UK. Each identified hazard had a basis that was backed by work undertaken by independent third parties and which had been peer reviewed. This data was used during the development of an on-line best practice guide with a scoring system that rewarded good and best processing practices and could be used by abattoir staff and/or inspection officers to evaluate particular slaughter processes. The system asked a series of questions concerning the processing practices, and scored the answers depending on the perceived influence on the hygienic quality of the meat product. Published information concerning each point in the slaughter process was available on-line to the users of the Guide.

The Project Leader and three other senior members of the research team attended a conference on 6th December 2007 at Chartridge Conference Centre, Chartridge, Chesham, Bucks HP5 2TU to discuss the draft Best Practice Guide developed in FSA project number MO1045, applied to poultry (and pig) slaughter lines. The meeting included other research workers from Bristol University and from other institutions, as well as the Project Officer from both MO1039 and MO1045, (Mrs Mary Howell) and her colleague Ms Vicky Inness, both from the Food Standards Agency. Each step was considered and its weighting with respect to its effect on the hygiene of the final product was agreed by consensus, based on the available data. At that time, the data concerning decontamination of poultry meat using physical and/or chemical methods had not been published, and could not therefore be used as evidence to support the Best Practice Guide. In addition, chemical decontaminants had not been approved for commercial use. Use of a decontamination step immediately before or after chilling was considered the most effective method of improving the safety of poultry meat. Data provided by the research team concerning the effect of steam or hot water treatment of poultry meat was included to support a question asking whether an 80°C hot water wash was employed by the users of the Best Practice guide.

The M01039 research team assisted with the provision of project outputs prior to publication. In addition, and in combination with the other assembled researchers, the MO1039 project team donated technical expertise by helping score the relative weights assigned to each step in the slaughter line, published information concerning poultry and pig processing, and with the relative weight assigned to each step in the slaughter line by the scoring system.

13 Overall project conclusions

This project was carried out to help the FSA meet its aim of reducing *Campylobacter* contamination of poultry.

The key scientific objectives of the project were to:

1. Identify a 'typical' poultry processing system and any features present in current lines that are not typical but are likely to influence contamination.

2. Quantify and identify the main contamination paths in current processing.

3. Develop methods of reducing contamination and cross-contamination.

4.Evaluate various intervention steps for reducing contamination and cross-contamination.

5. Identify the key scientific data that could be used to develop a best practice guide.

Data from this project was regularly fed into FSA Project M01045 (Evaluation of data generated by meat plants for current HACCP verification purposes and future slaughterhouse hygiene purposes).

Our results were as follows:

13.1 Objective 1: Identify a 'typical' poultry processing system and any features present in current lines that are not typical but are likely to influence contamination

Six chicken, two turkey and one duck processing line were surveyed and a report written. In each case the project team followed the production line from lairage to portion cutting, and then interviewed production and management staff. The information gained was used to inform continuing studies of current industrial practice and allow targeting of more detailed experimental evaluative measurements. The results from all chicken plants were further combined into an anonymous description of a 'typical' UK chicken processing plant. There were deemed to be too few turkey and duck plants to form a representative sample. Where applicable the 'typical' chicken plant was contrasted to the turkey and duck lines. The survey also included an assessment of hygiene, disinfection and cleaning regimes, on which a separate report was written. The effectiveness of the most commonly-used commercial disinfectants was tested against a panel of Campylobacter isolates and other bacteria.

The abattoir survey revealed that the techniques used in all chicken processing plants were similar, and that the cleaning the disinfection methods were effective against Campylobacters. However, because poultry processing is highly mechanised and is conducted at speeds up to 12,000 carcasses per hour, effective cleaning and disinfection cannot be carried out between flocks, only between shifts – overnight or over the weekend. Also, cross-contamination between adjacent carcasses on the line is unavoidable, and occurs via the machinery and also by direct contact.

13.2 Objective 2: Quantify and identify the main contamination paths in current processing

Extent of cross-contamination from C+ to C- carcasses. This was first investigated by examining neck skins and caecal contents taken at random from all flocks processed over a number of days in two poultry processing plants. The caecal contents were examined in order to know whether the flocks were C+ or C-. The neck flaps from C- flocks processed after C+ flocks were then examined for numbers of campylobacters. Numbers of Campylobacter on neck flaps from some C+ flocks were also determined for comparison. Results showed that numbers on neck flaps from C- flocks were almost always <25 cfu g^{-1}, (160/170 were <25 cfu

g^{-1}, seven between 25 and 99 cfu g^{-1} and three between 100 and 999 cfu g^{-1}) whereas those from neck flaps from C+ flocks were significantly higher (of 105 examined, two (2%) contained <25, ten (9.5%) between 25 and 99, 49 (46.5%) between 100 and 999 and 43 (42%) 1000 or more).

A further investigation was then carried out to determine whether the first few carcasses from the C- flocks carried higher numbers of campylobacters than those observed in the previous survey. The experiment was therefore repeated, taking five neck skins from the first ~100 carcasses processed, five from carcasses ~500-600 and five from carcasses ~5000-5100 of all flocks processed over several days and from two different poultry plants. Four C- flocks processed after C+ flocks were identified and the numbers of campylobacters per g neck skin compared with those obtained from carcasses at the same points during processing of C+ flocks. After the first ~100 carcasses, almost all the carcasses from C- flocks had <25 cfu campylobacters g^{-1} neck skin, while numbers on neck flaps of carcasses from C+ flocks remained high throughout 28/56 (50%) exceeding 100 cfu g^{-1}, and 10/56 (18%) exceeding 1000 cfu g^{-1}.

Numbers of *Campylobacter* spp. transferred from Campylobacter positive chickens to their carcasses during processing. Visits were made by a team of eight to chicken processing plants on five occasions. Ten samples of necks or neck skins were taken at each of six points on the line during the processing of four flocks at each visit, and numbers of campylobacters, Enterobacteriaceae capable of multiplying at 41.5°C, and pseudomonads were enumerated. Enterobacteriaceae were included as indicators of campylobacter contamination, as they were found in similar numbers in the intestine, and not all flocks were colonised with campylobacters. Pseudomonads were an index of contamination that occurred from the processing environment. Most carcass contamination with *Campylobacter* spp. and Enterobacteriaceae were detected after scalding with little obvious increase after plucking, or after evisceration. Contamination with pseudomonads increased steadily all down the line after scalding.

In order to clarify whether process was the most important source of carcass contamination with campylobacters, batches of chicken carcasses were removed from the line immediately after plucking and dipped in water at 80°C for 20 seconds before replacement on the evisceration line. Control carcasses (processed normally) were taken after evisceration, as well as carcasses that had been decontaminated with hot water after plucking. All were sampled by examination of neck flaps or necks and the carcass rinse method. Results showed that plucking and evisceration contributed to a similar degree to numbers of *Campylobacter* spp. and Enterobacteriaceae on the fully processed carcasses.

13.3 Objective 3: Develop methods of reducing contamination and cross-contamination

Brain-storming sessions and discussions were held in order to identify the methods most likely to be successful in reducing numbers of campylobacters on carcasses from Campylobacter-positive (C+) and Campylobacter-negative (C-) flocks.

With C+ flocks the problem is to try to minimise transfer to the finished carcass, of campylobacters present in high numbers in the intestinal contents and on the feathers of the birds. Practical investigations in processing plants showed that similar proportions of contamination was occurring at two main points - during the scald and pluck stage, and subsequently during the evisceration steps. It was therefore concluded that reducing significantly the numbers of campylobacters reaching the carcasses during scalding and plucking would be of little benefit if subsequent processing steps during evisceration

contributed almost as many. However, a better system for scalding and plucking and/or cleaning and disinfection all along the line between flocks might be effective in reducing cross-contamination from C+ to C- flocks. It was therefore decided to investigate in more detail how many campylobacters were transferred to C- carcasses when processed immediately after C+ carcasses. If this was significant, it would justify introducing a cleaning step between flocks, or scheduling C+ flocks to be slaughtered after C- flocks.

The other clear possibility was to investigate the effect of end-product treatment of the fully-processed carcasses, either immediately before, during or after chilling. The possibilities were to use physical (e.g. steam at atmospheric pressure or dipping in hot water) or chemical (e.g. chlorine, chlorine dioxide, acidified sodium chlorite, ozone, trisodium phosphate, mixtures of peroxy acids).

13.4 Objective 4: Evaluate various intervention steps for reducing contamination and cross-contamination

Cross-contamination from campylobacter positive to campylobacter-negative carcasses only occurred on the first few hundred carcasses, and in relatively low numbers. Cleaning and disinfection of the machinery between flocks would reduce cross-contamination to a negligible level, but would not be practicable, and would have no effect on carcasses from campylobacter-positive flocks.

The plucking and subsequent evisceration process both contribute significantly to the numbers of campylobacters on carcasses, but decontamination of carcasses immediately post pluck did not result in clearly lower numbers of campylobacters when examined after evisceration. This indicated that evisceration negated the beneficial effect of post-puck decontamination. Therefore, most effective measure would be to use a physical or chemical treatment immediately before chilling. Heat treatment using steam or hot water had previously been found to reduce numbers of campylobacters significantly on the outside of poultry carcasses (Corry *et al*., 2007; James *et al*., 2007) so several chemicals were evaluated. It was decided that investigations should be carried out as far as possible on typical carcasses during normal production. Only naturally contaminated carcasses taken off the line before chilling and used as soon as possible would be used in any laboratory experiments. The effect of acidified sodium chlorite (ASC), chlorine dioxide (CD), peroxyacetic acid (PAA) and tri-sodium phosphate (TSP) on naturally occurring *Campylobacter*, *Enterobacteriaceae* and *Pseudomonas* spp. on the breast and neck skin of chicken carcasses was compared.

For analysis, the results were subdivided into six microbe-type/skin-location combinations with each subdivision ranked by: a) cfu remaining after treatment, b) mean reductions, and c) the proportional change in numbers of samples below the limit of detection (LoD).

The three groups of bacteria responded similarly to the chemicals applied. *Campylobacter* spp. were no more susceptible than the other two groups. No single chemical treatment gave the best effect across all subdivisions and ranking methods. Generally, ASC and TSP performed better than PAA with CD and water washing alone having little effect. A 30 second chemical treatment was usually more effective than a 15 second treatment. Where only a short (15 second) spray time was possible, ASC was the most effective. If longer treatments were possible, TSP was the most effective choice. Rinsing with water reduced the effectiveness of the chemicals.

13.5 Objective 5: Identify the key scientific data that could be used to develop a best practice guide

While some improvements could be achieved in terms of numbers of Campylobacters on the final product by improving hygiene at various points on the line (e.g. **scalding**: temperature, serial scald tanks, point of addition of fresh water; **plucking**: hot water sprays, post pluck wash; **evisceration**: careful adjustment of machinery, spray washes, minimal contact of carcasses with machinery; **Inside/outside wash:** design of sprays, volume of water per carcass; **(Air) chilling:** air temperature, provision of sprays, speed and direction of cold air, arrangement of carcass conveyors), the most effective measure would be to use a physical or chemical treatment immediately before, during or after chilling. The plucking process causes carcasses from Campylobacter-positive flocks to become highly contaminated with Campylobacters. Decontamination after plucking and before evisceration would not be useful because evisceration also contaminates the carcasses.

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15 Project publications

15.1 Published papers

H. S. Musavian, N. H. Krebs, U. Nonboe, **J E.L. Corry** and **G. Purnell** 2014 Combined steam and ultrasound treatment of broilers at slaughter: A promising intervention to significantly reduce numbers of naturally occurring campylobacters on carcasses. International Journal of Food Microbiology, **176** 23 – 28.

Purnell, G, James C, James SJ, Howell, M. and **Corry, J E L** 2013 Comparison of acidified sodium chlorite, chlorine dioxide, peroxyacetic acid and tri-sodium phosphate spray washes for decontamination of chicken carcasses. Food and Bioprocess Technology, doi: 10.1007/s11947-013-1079-7

J.J. Sun 2006 **"**Investigation of disinfectant resistance of thermophilic campylobacters and *Pseudomonas* spp. found in poultry processing plants." MSc thesis, University of Bristol.

Ana Hermosilla 2004 "Transfer of contamination of *Campylobacter* spp. from positive broiler flocks to negative flocks during processing." MSc thesis, University of Bristol.

15.2 Poster papers

J.E.L. Corry, G. Purnell, C. James, R. Pinho, A. Hedges, F. Jorgensen[3], S. J. James, M. Howell, 2008 Evaluation of chemicals for the inactivation of naturally occurring thermophilic *Campylobacter* spp. on poultry carcasses. FoodMicro 2008, Aberdeen, Scotland.

J.E.L. Corry, C. S. Barbedo-Pinto, J. Cestra, F. Jorgensen, L. Williams, C. James[3], S. J. James, G. Purnell, M. Howell 2007 Investigation of optimum intervention points to minimise transfer of *Campylobacter* spp. from the live chicken to the carcass during processing. CHRO 2007, Rotterdam, The Netherlands.

A.M. Hermosilla, N. Fincham, V.M. Allen J.E.L. Corry 2005 Transfer of contamination. from Campylobacter-positive broiler flocks to Campylobacter-negative flocks during processing. CHRO 2005, Goldcoast, Australia.

16 Glossary

AA: Acetic acid.

ASC: Acidified sodium chlorite.

ATSDR: Agency for Toxic Substances and Disease Registry, USA.

APC: Aerobic Plate Count.

bw: Body weight.

CAS: Chemical Abstracts Service.

CD: Chlorine dioxide.

CPC: Cetylpyridinium chloride, or 1-hexa-decyl pyridinium chloride.

CTC: Chlortetracycline.

EFSA: European Food Safety Authority

US-EPA: United States Environmental Protection Agency.

HEDP: 1-Hydroxyethylidene-1,1-diphosphonic acid.

HP: Hydrogen peroxide.

EA: Eau Activée®, active product of Catallix® lactoperoxidase system.

IARC: International Agency for Research on Cancer.

IPCS: International Programme on Chemical Safety of the World Health Organization.

LOD: Limit of detection.

LPS: Lactoperoxidase systems.

FSANZ: Food Standards Australia New Zealand.

LOAEL: Lowest observed (adverse) effect level - the lowest dose level in a study with experimental animals at which a(n) (adverse) health effect was observed.

MOS: Margin of safety.

NOAEL: No observed (adverse) effect level - the highest dose level in a study with experimental animals at which no (adverse) health effects were observed.

OA: Octanoic acid.

PA: Peroxyacid.

PAA: Peroxyacetic acid.

POA: Peroxyoctanoic acid.

RfC: Reference concentration - An estimate of the daily inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (definition of the EPA).

RfD: Reference dose - An estimate of the daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (definition of the EPA).

SCTEE: Scientific Committee on Toxicity, Ecotoxicity and the Environment of the EC.

SCVPH: EC Scientific Committee on Veterinary measures relating to Public Health.

TCA: Tolerable concentration in air - An estimate of the concentration in air to which one can be exposed daily during lifetime without adverse health effects.

TDI: Tolerable daily intake - An estimate of the daily dose that can be taken daily during lifetime by the oral route without adverse health effects.

TSP: Trisodium phosphate.

UDS: Unscheduled DNA synthesis.

USDA: United States Department of Agriculture.

US-FDA: United States Food and Drug Administration.

WHO: World Health Organization.

JECFA: Joint FAO/WHO Expert Committee on Food Additives and Contaminants.

THM: Trihalomenthane.

HAA: Haloacetic Acid.

ORP: Oxidation–reduction potential.

EDTA: Ethylenediaminetetraacetic acid.

LPS: Lipopolysaccharide.

GRAS: Generally Recognised As Safe.

LAB: Lactic acid bacteria.

FAO: Food and Agriculture Organization of the United Nations.

WHO: World Health Organization.