final report

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In-plant evaluation of oxidant-based application during spray chilling as an antimicrobial intervention for beef carcases – follow-up trials

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In-plant evaluation of oxidant-based application during spray chilling as an antimicrobial intervention for beef carcases – follow-up trials

Background

- The University of Tasmania (UTas) has been working on application of an oxidant during spray chilling as an antimicrobial intervention for beef carcases.
- In our previous trials at the JBS Longford abattoir (conducted in September-October 2016), UTas added oxidants (*i.e*., peroxyacetic acid and chlorine dioxide separately) to spray chilling water and evaluated their effectiveness in improving the microbiological quality of carcases.
- The results of those trials indicated that such applications during chilling have the potential to greatly improve the microbiological quality of carcases (based on total viable count, and levels of coliforms and generic *E. coli*). The data for TVC highlight that the test application achieved at least a 0.96-log reduction (on average). However, its effectiveness could not be accurately and reliably determined due to high variation in levels of target bacteria (*i.e.,* coliforms and *E. coli*).
- To this end, further trials using both uninoculated and inoculated carcases were carried out to more accurately determine the effectiveness of the test application. The data generated from these trials would provide an indication of its performance as an antimicrobial intervention in commercial settings as well as facilitating its commercial adoption.

Approach

- All trials were conducted at the JBS processing plant in Longford (TAS) between May and June 2017.
- These trials involved use of carcases (either uninoculated carcases or carcases previously inoculated with non-pathogenic *E. coli*), and a commercial chiller to simulate an industrial application.

Trials involving use of carcases inoculated with non-pathogenic E. coli

- Three separate trials were carried out over two consecutive weeks. A total of three sides (or carcases) were randomly selected and used in each trial.
- A five-strain cocktail of non-pathogenic *E. coli* (*i.e.,* EC1604, EC1605, EC1606, EC1607, and EC1608) were prepared in 0.1% bacteriological peptone to obtain an inoculum containing approximately 10^{-6-7} CFU/ml. It should be noted that all of these *E. coli* strains are found to contain no known virulence markers for pathogenic *E. coli* and which, between them, have characteristics very similar to various isolates of *E. coli* O157:H7.
- Four different sites of each carcase were carefully painted with the bacterial inoculum. These include hind leg, flank, fore leg and neck. All inoculated carcases were then left to dry for approximately 20 min to allow attachment of the bacteria to the inoculated surface.
- The inoculated carcases were subjected to the process of spray chilling. In all trials, the same spray chilling protocol was used, which involves a 30-s spray every 15 min for a total of 42 sprays. Untreated carcases were sprayed with water (as normally occurs during spray chilling), while treated carcases were sprayed with water from the same source but containing either peroxyacetic acid (PAA at 200 ppm) or chlorine dioxide (ClO_2 at 50 ppm).

- To determine changes in *E. coli* numbers due to spray chilling (either with water or an oxidant solution), an area (measuring approximately 10 X 10 cm) at each inoculated site was swabbed before commencing the chilling process, at 20 h (to simulate overnight chilling), and at 50 h and 60 h (to simulate weekend chilling) of chilling.
- An enumeration method based on Petrifilm was then performed on swab samples to determine *E. coli* numbers (expressed as $\log_{10}$ CFU/cm^2). Log reduction in *E. coli* numbers at any given time point was calculated based on the differences in log units between untreated carcases and carcases treated with an oxidant.

Trials involving use of uninoculated carcases

- In addition to the above trials with inoculated carcases, three other trials were carried out over three consecutive weeks using uninoculated carcases. A total of 50 carcases were randomly selected and used in each trial. It also should be noted that 25 additional carcases were included in the first trial to obtain the baseline data for *E. coli* prevalence and numbers on carcases prior to the process of spray chilling (*i.e.,* hot carcases)
- In all trials, the test carcases were subjected to the same spray chilling protocol, as described above. However, untreated carcases were sprayed with water (as normally occurs during spray chilling), while treated carcases were sprayed with water from the same source but containing either PAA (at 120 ppm) or ClO_2 (at 80 ppm).
- To determine changes in *E. coli* prevalence and numbers due to the process of spray chilling (either with water or oxidant solutions), five different sites on each of 25 test carcases (n=125) were swabbed before commencing the chilling process (0 h or baseline data; for the first trial only), at 20 h and 50 h of chilling. Test sites near the hide-opening cutting line were selected, including inside hind leg (measuring approximately 500 cm^2), bung (300 cm^2), flank (500 cm^2), brisket (500 cm^2) and neck (1,000 cm^2).
- Enumeration of *E. coli* was performed on swab samples by two different methods: Petrifilm-based method for determination of *E. coli* **prevalence** and Most Probable Number (MPN)-based technique for *E. coli* **prevalence** and **numbers**. The results are reported as the percentage of positive samples for *E. coli* and/or expressed as CFU/100 cm^2.

Key results

Trials using carcases inoculated with non-pathogenic E. coli

- Figures 1-4 describe changes in *E. coli* numbers on inoculated carcases during exposure to the chilling process and when subjected to the application of an oxidant (either PAA at 200 ppm or ClO_2 at 50 ppm).

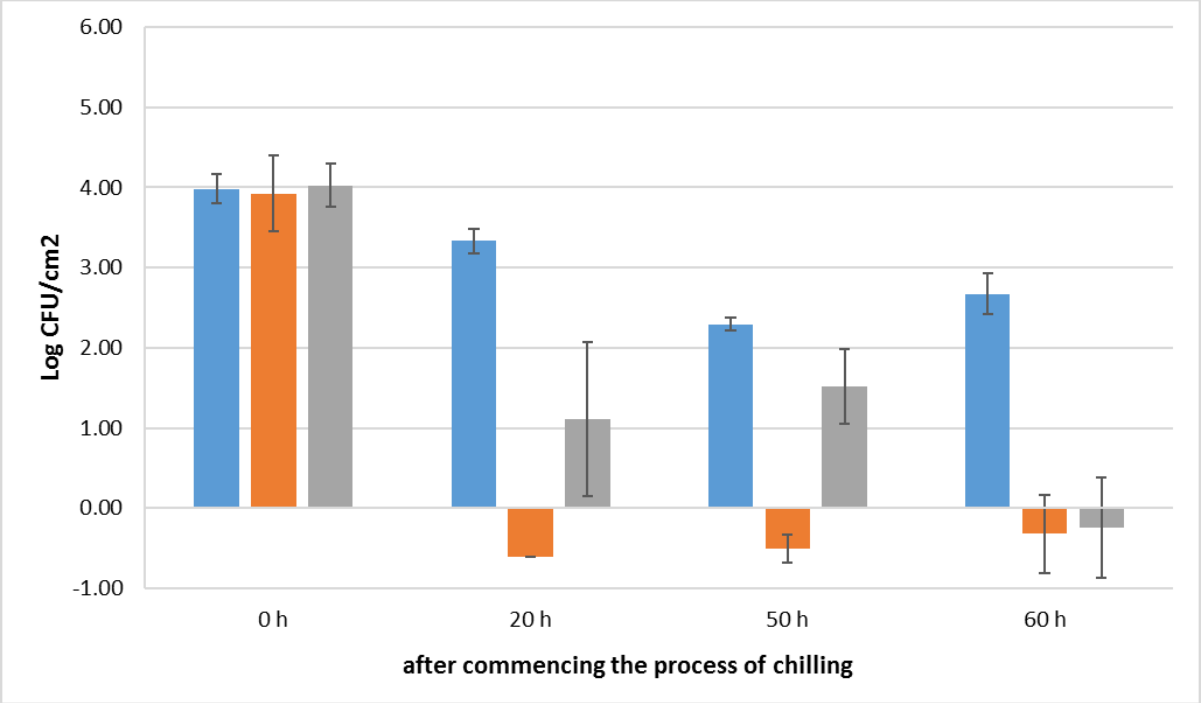

Figure 1. Population changes of a five-strain of non-pathogenic *E. coli* on the hind leg of artificially contaminated carcases during the process of spray chilling with water (control; blue), peroxyacetic acid (at 200 ppm; orange) and chlorine dioxide (at 50 ppm; grey). The detection limit is -0.60 log CFU/cm^2.

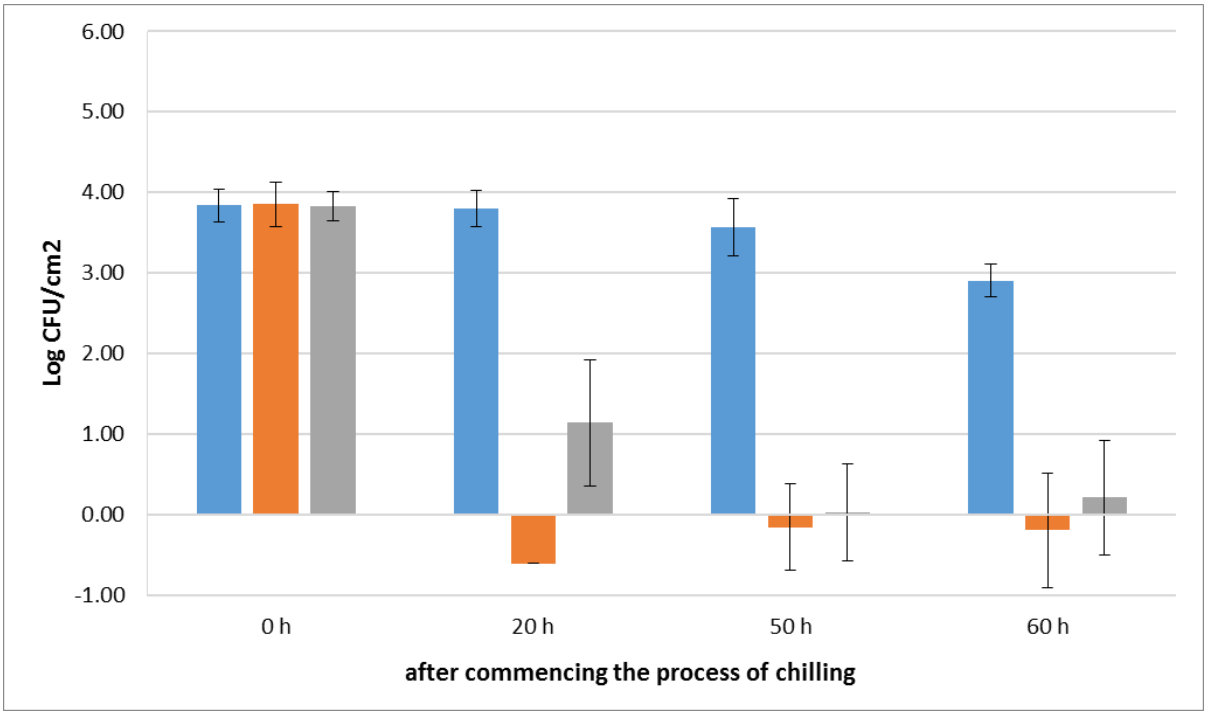

Figure 2. Population changes of a five-strain of non-pathogenic *E. coli* on the flank of artificially contaminated carcases during the process of spray chilling with water (control; blue), peroxyacetic acid (at 200 ppm; orange) and chlorine dioxide (at 50 ppm; grey). The detection limit is -0.60 log CFU/cm^2.

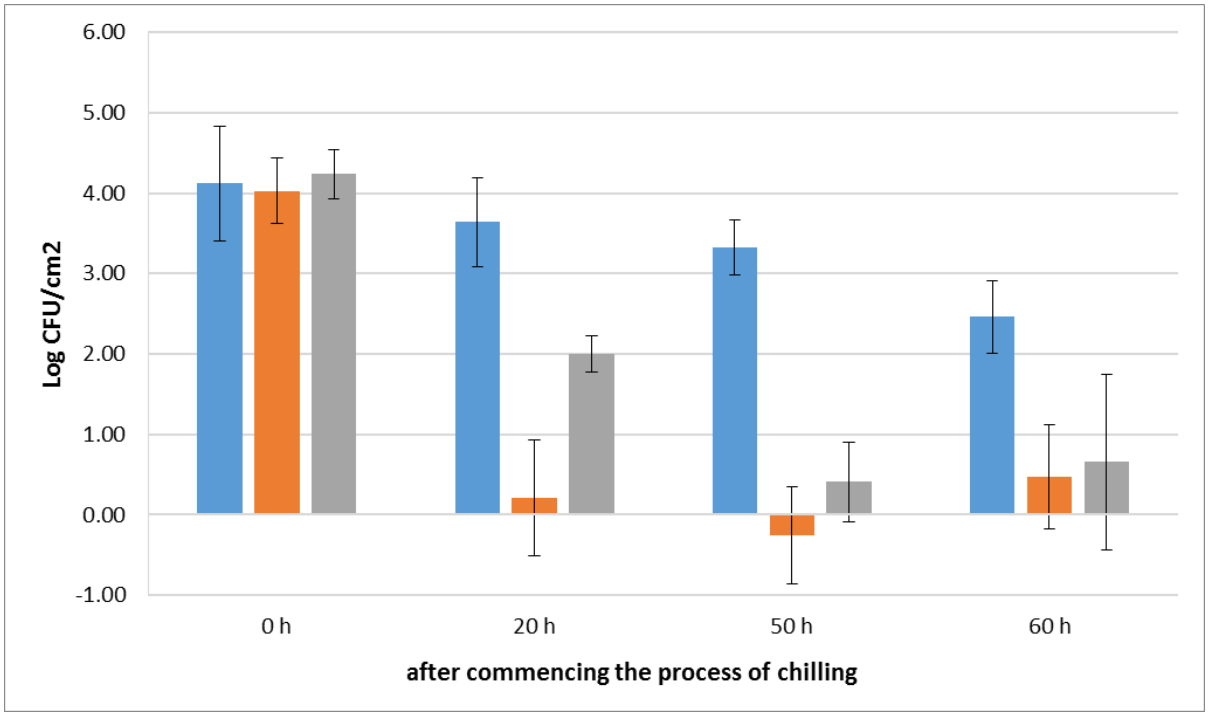

Figure 3. Population changes of a five-strain of non-pathogenic *E. coli* on the fore leg of artificially contaminated carcases during the process of spray chilling with water (control; blue), peroxyacetic acid (at 200 ppm; orange) and chlorine dioxide (at 50 ppm; grey). The detection limit is -0.60 log CFU/cm^2.

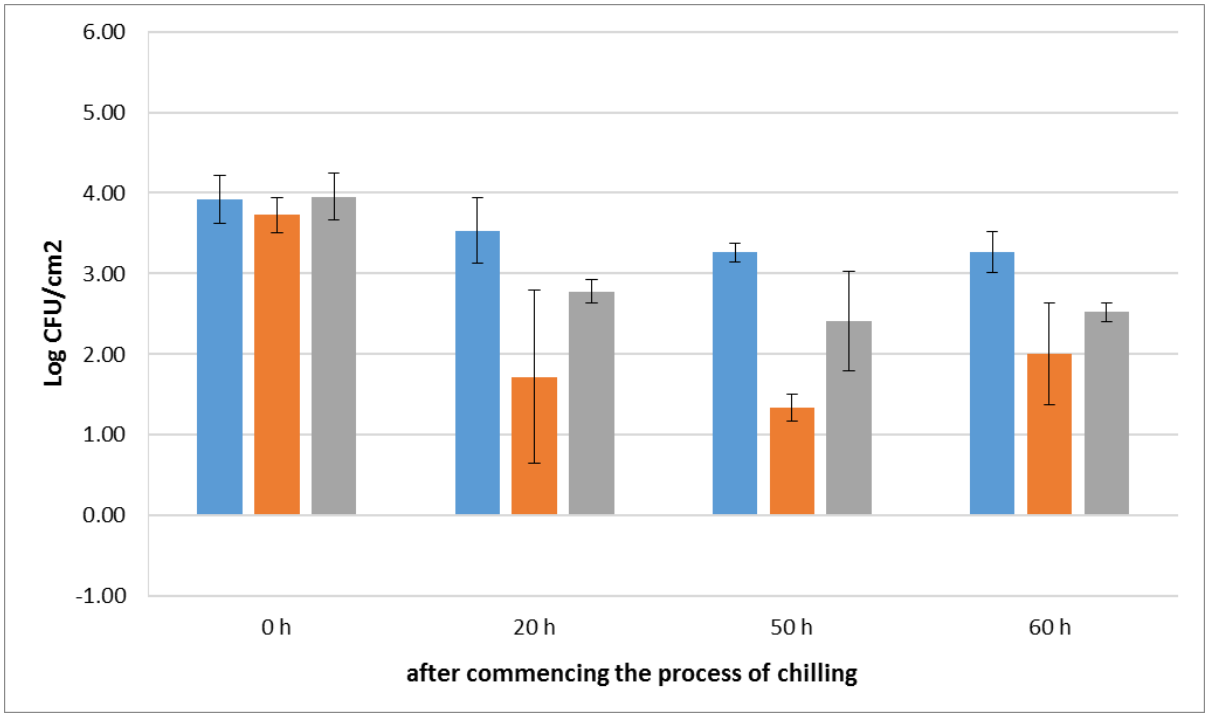

Figure 4. Population changes of a five-strain of non-pathogenic *E. coli* on the neck of artificially contaminated carcases during the process of spray chilling with water (control; blue), peroxyacetic acid (at 200 ppm; orange) and chlorine dioxide (at 50 ppm; grey). The detection limit is -0.60 log CFU/cm^2.

- *E. coli* numbers at the inoculated sites were found to range from 3.7 to 4.9 log CFU/cm^2 before commencing the process of chilling (*i.e.*, at 0 h; Figures 1 to 4). There were no systematic differences in the numbers of *E. coli* among those sites and between trials.
- It was evident in all cases with the exception of neck that the normal process of spray chilling (*i.e.,* sprayed with water only) reduced *E. coli* numbers over the course of the trials (Figures 1-4). An approximately 1-log reduction (on average) in *E. coli* numbers was observed at 60 h of chilling (when compare to those numbers obtained before commencing chilling). This reinforces the idea that the process of spray chilling alone can reduce *E. coli* numbers.
- When compared to *E. coli* numbers on the untreated carcases, application of any of the test oxidants caused a reduction in the numbers of *E. coli* at all time points (Figures 1-4). Table 1 summarises log reduction in *E. coli* numbers at each inoculated sites due to the application of an oxidant during spray chilling.

Table 1. Log reduction in *E. coli* numbers at different sites of artificially contaminated carcases due to the application of an oxidant during spray chilling.

Time after commencing the chilling process	**Log reduction in *E. coli* numbers (CFU/cm^2)**[a,b]	
	PAA application (at 200 ppm)	**ClO_2 application (at 50 ppm)**
Hind leg		
20 h	>3.93 ± 0.16	2.22 ± 0.97
50 h	>2.80 ± 0.19	0.77 ± 0.47
60 h	>2.99 ± 0.55	>2.92 ± 0.67
Flank		
20 h	>4.40 ± 0.22	2.66 ± 0.82
50 h	3.72 ± 0.64	>3.53 ± 0.70
60 h	>3.10 ± 0.74	>2.69 ± 0.74
Fore leg		
20 h	>3.44 ± 0.91	1.64 ± 0.59
50 h	>3.58 ± 0.69	2.91 ± 0.60
60 h	1.98 ± 0.79	>1.81 ± 1.18
Neck		
20 h	1.81 ± 1.15	0.75 ± 0.43
50 h	1.93 ± 0.20	0.85 ± 0.62
60 h	1.27 ± 0.68	0.75 ± 0.28

a. Log reduction in *E. coli* numbers at any given time point was calculated based on the differences in log units between untreated carcases and carcases treated with an oxidant.

b. Values with a 'greater than' symbol (>) indicate at least one sample within the treatment had counts below the detection limit (-0.60 log CFU/cm^2).

- The data revealed that application of PAA and ClO_2 reduced *E. coli* numbers after commencing the chilling process for 20 h. Thereafter, no further effects were observed (Figures 1-4 and Table 1).
- In the present trials, application of PAA appeared to be more effective against *E. coli* at all inoculated sites when compared to ClO_2 application (Figures 1-4 and Table 1). However, both PAA and ClO_2 applications produced different antimicrobial effects at different sites. The highest reduction in *E. coli* numbers was observed on flank (>4.40-log and 2.66-log reduction on average for application of PAA and ClO_2, respectively), while neck was the least effective site (1.81- and 0.75-log reduction for PAA and ClO_2, respectively). These observations may be explained by the fact that not all parts of carcases are equally exposed to spray during chilling. Full coverage of

carcases with an oxidant solution during spray chilling is, therefore, required to achieve its maximum efficacy.

Trials using uninoculated carcases

- Figure 5 describes changes in the overall prevalence of generic *E. coli* on carcases during exposure to the spray chilling process and when subjected to the application of an oxidant (either PAA at 120 ppm or ClO_2 at 80 ppm), as determined by Petrifilm-based method. It should be noted that the detection limit of this enumeration method is 5 CFU/100cm^2 for hind leg, flank and brisket, 8 CFU/100cm^2 for bung, and 3 CFU/100cm^2 for neck.

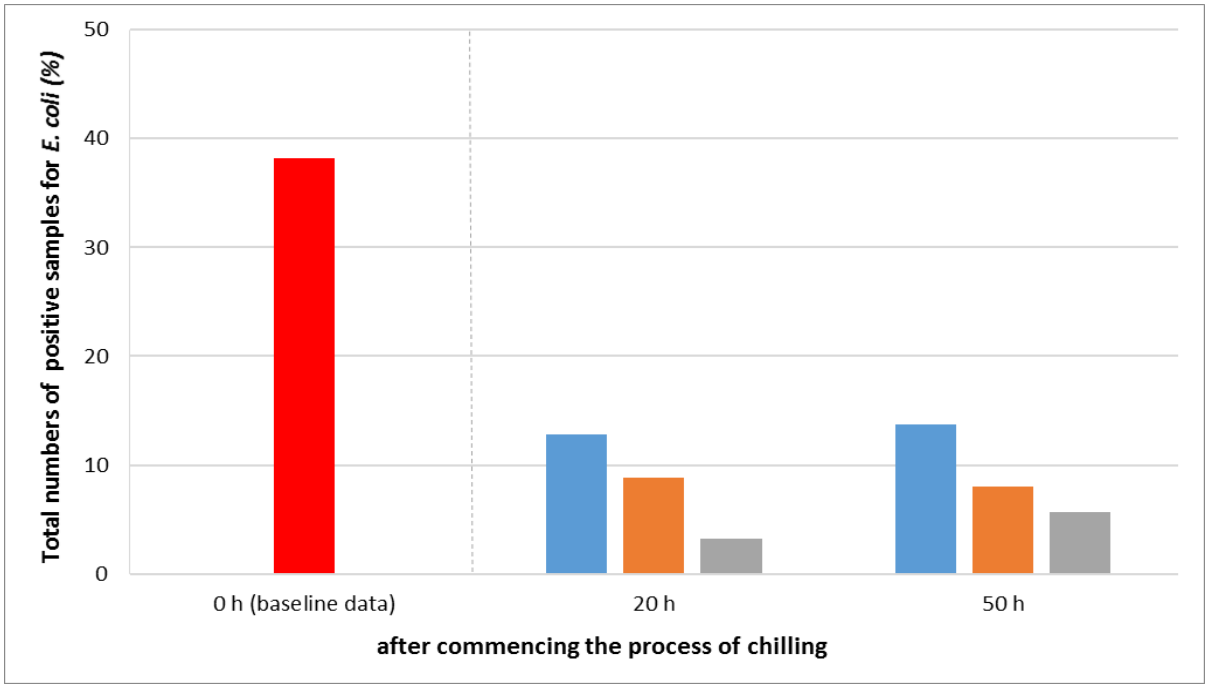

Figure 5. Changes in the overall prevalence of generic *E. coli* (%) on carcases prior to (baseline data; red) and during the process of spray chilling with water (control; blue), peroxyacetic acid (at 120 ppm; orange) and chlorine dioxide (at 80 ppm; grey), as determined by Petrifilm-based method. The detection limit is 5 CFU/100cm^2 for hind leg, flank and brisket, 8 CFU/100cm^2 for bung, and 3 CFU/100cm^2 for neck.

- *E. coli* prevalence appeared to be relatively high before commencing the process of chilling (Figure 5). Approximately 40% of all samples tested (n=125) were positive for *E. coli*. This level of *E. coli* prevalence is as expected due to the sampling from hot carcases and large area of high contamination sites (*i.e.,* those near hide-opening cutting line).
- The prevalence of *E. coli* reduced dramatically to 13%, 9% and 3% following the process of spray chilling with water, PAA and ClO_2 solution for 20 h, respectively (Figure 5). No further effects were then observed until the end of the trials. These results highlight that not only the process of spray chilling alone could reduce *E. coli* prevalence, but also the test application was more effective against *E. coli*.
- To further evaluate the effectiveness of the test application, an MPN-based method was performed to provide more accurate determination of *E. coli* prevalence and numbers. This enumeration method has a lower detection limit than that of the method based on Petrifilm. It is

estimated that the detection limit of the MPN method is 0.3 CFU/100cm^2 for hind leg, flank and brisket, 0.6 CFU/100cm^2 for bung, and 0.2 CFU/100cm^2 for neck. Figures 6-10 describe changes in the percentage of positive samples characterized based on *E. coli* numbers at different test sites, as determined by MPN-based method.

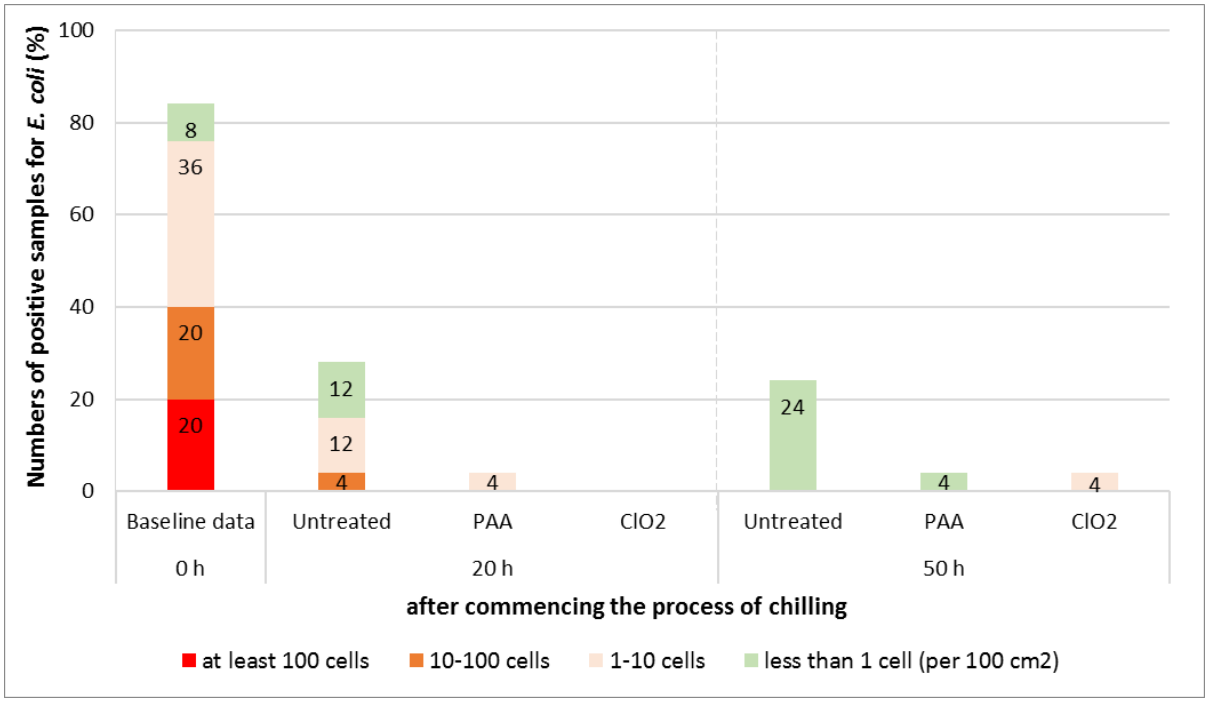

Figure 6. Changes in the prevalence of generic *E. coli* (%) on the hind leg of carcases prior to (baseline data) and during the process of spray chilling with water, peroxyacetic acid (PAA at 120 ppm) and chlorine dioxide (ClO_2 at 80 ppm), as determined by MPN-based method**.** The prevalence data were further characterized based on *E. coli* numbers (CFU/100cm^2). The detection limit is 0.3 CFU/100cm^2.

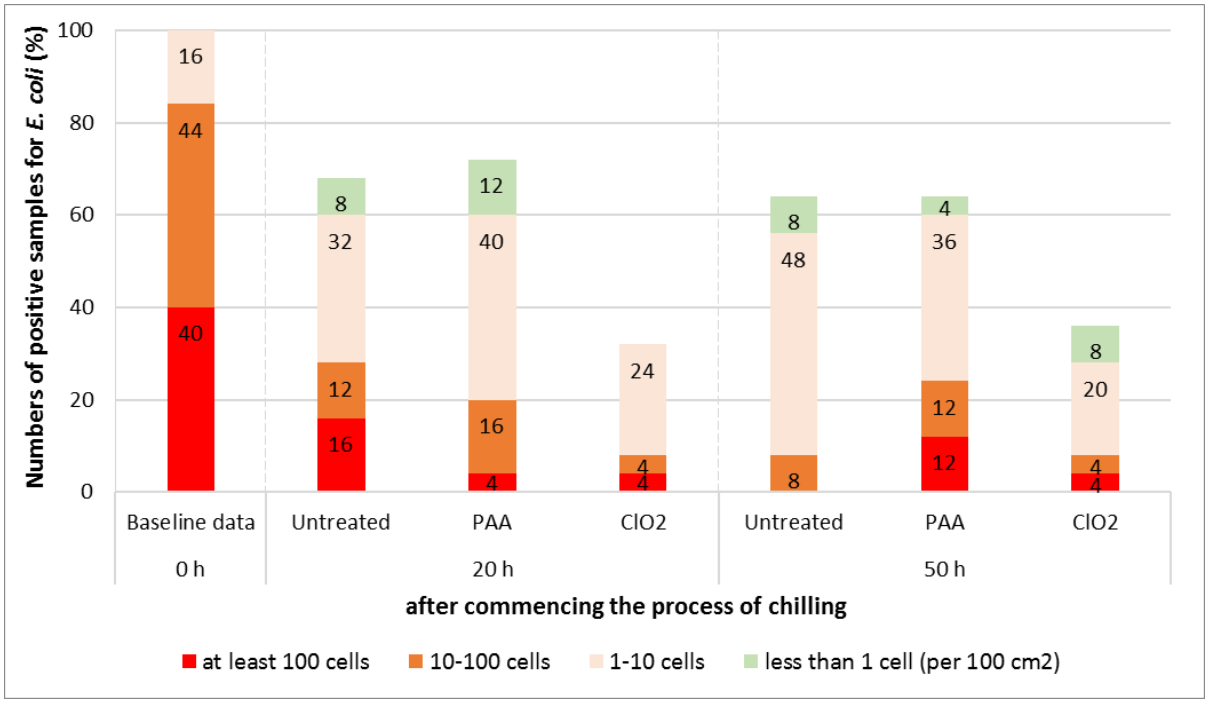

Figure 7. Changes in the prevalence of generic *E. coli* (%) on the bung of carcases prior to (baseline data) and during the process of spray chilling with water, peroxyacetic acid (PAA at 120 ppm) and chlorine dioxide (ClO_2 at 80 ppm), as determined by MPN-based method. The prevalence data were further characterized based on *E. coli* numbers (CFU/100cm^2). The detection limit is 0.6 CFU/100cm^2.

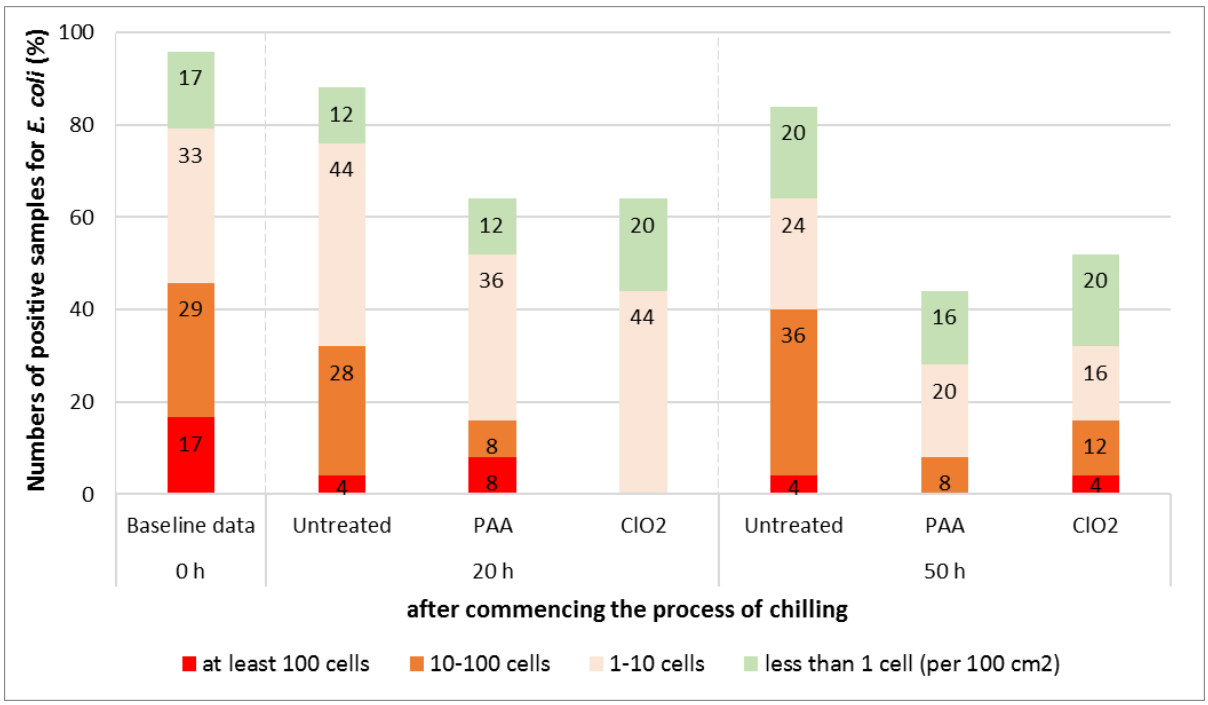

Figure 8. Changes in the prevalence of generic *E. coli* (%) on the flank of carcases prior to (baseline data) and during the process of spray chilling with water, peroxyacetic acid (PAA at 120 ppm) and chlorine dioxide (ClO_2 at 80 ppm), as determined by MPN-based method. The prevalence data were further characterized based on *E. coli* numbers (CFU/100cm^2). The detection limit is 0.3 CFU/100cm^2.

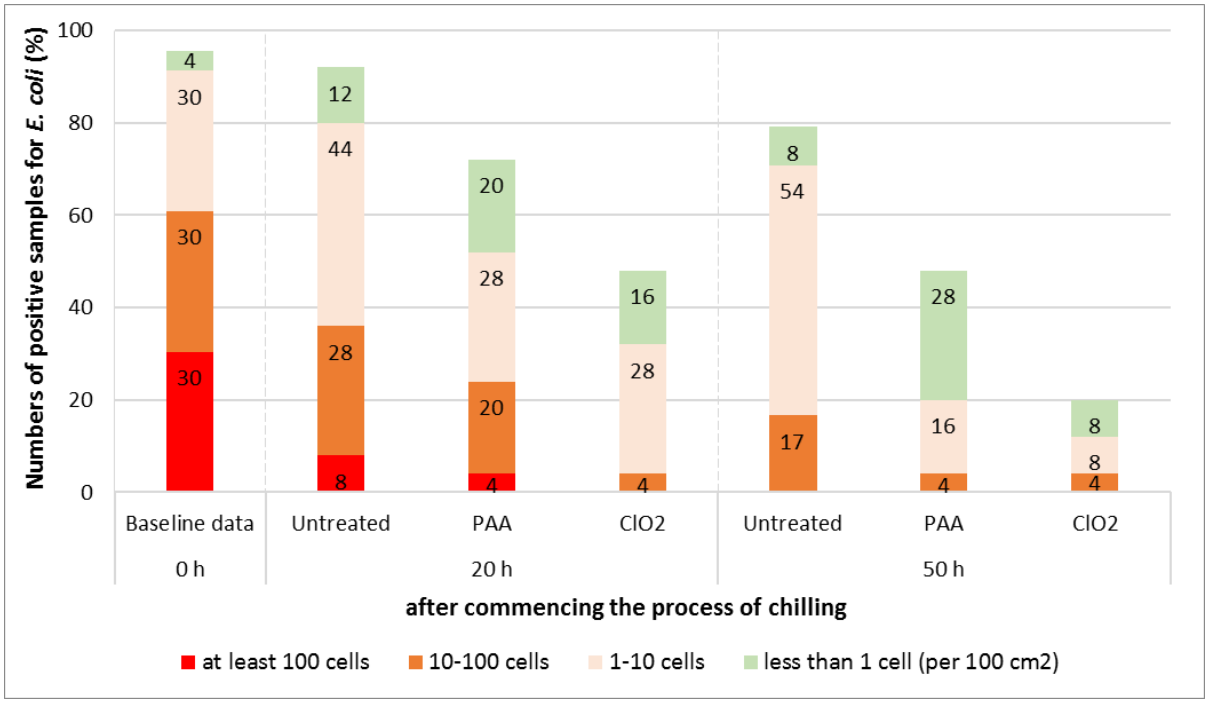

Figure 9. Changes in the prevalence of generic *E. coli* (%) on the brisket of carcases prior to (baseline data) and during the process of spray chilling with water, peroxyacetic acid (PAA at 120 ppm) and chlorine dioxide (ClO_2 at 80 ppm), as determined by MPN-based method. The prevalence data were further characterized based on *E. coli* numbers (CFU/100cm^2). The detection limit is 0.3 CFU/100cm^2.

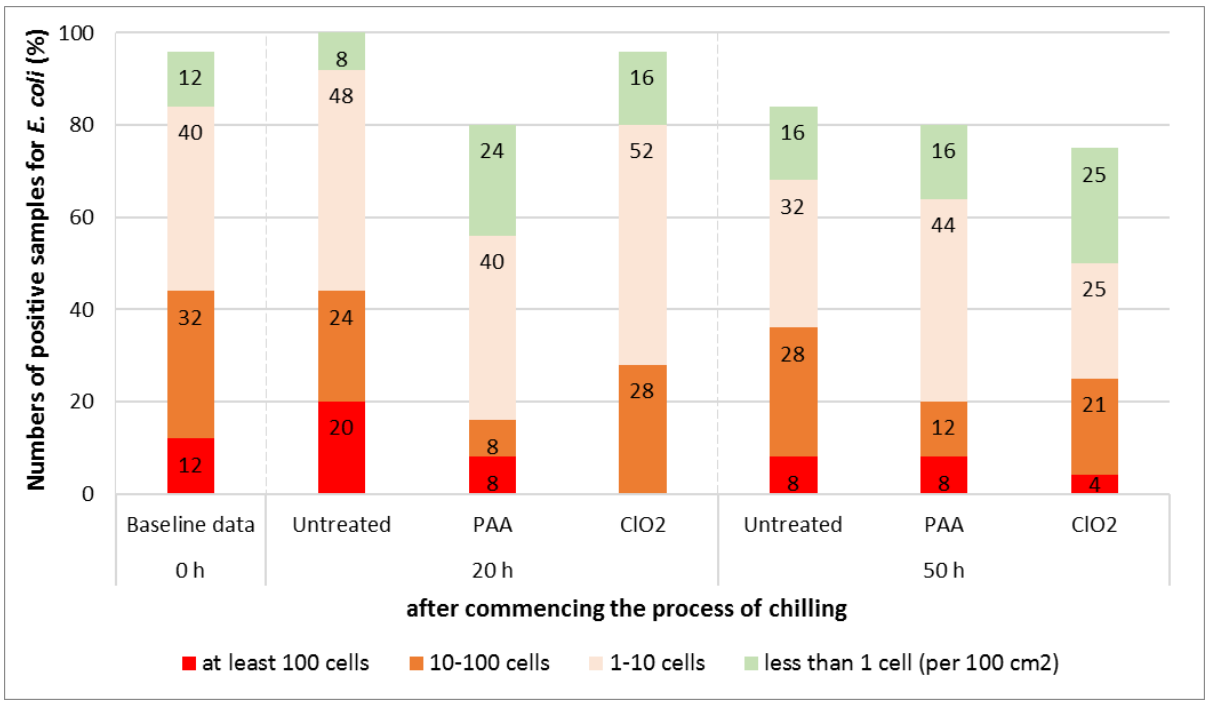

Figure 10. Changes in the prevalence of generic *E. coli* (%) on the neck of carcases prior to (baseline data) and during the process of spray chilling with water, peroxyacetic acid (PAA at 120 ppm) and chlorine dioxide (ClO_2 at 80 ppm), as determined by MPN-based method. The prevalence data were further characterized based on *E. coli* numbers (CFU/100cm^2). The detection limit is 0.2 CFU/100cm^2.

- The MPN data revealed a much higher prevalence of *E. coli* when compared to the data obtained from the Petrifilm method (as would be expected due to the much lower limit of detection) (Figures 6-10). At least 84% of the samples (n=25) was found positive for *E. coli* at each site of carcases before commencing the chilling process. Among those sites, bung was amongst the most highly contaminated with 84% of the samples containing at least 10 CFU/100cm^2. This is in contrast to hind leg, which was the least contaminated site with 40% of the samples containing at least 10 CFU/100cm^2.
- At all sites, application of both PAA and ClO_2 during spray chilling generally achieved a reduction in *E. coli* prevalence and numbers when compared to the normal process of spray chilling (*i.e.,* sprayed with water alone) (Figures 6-10). This observation is consistent with the data obtained from the Petrifilm method (*see* above) and further supports that the test application during spray chilling was more effective against *E. coli* on carcases.
- Despite the above observations, the antimicrobial effects of PAA and ClO_2 appeared to vary between the test sites (Figures 6-10). Both PAA and ClO_2 application was most effective against *E. coli* on hind leg (up to 4% of the samples contained no more than 10 CFU/100 cm^2), and least effective on neck (at least 16% of the samples contained at least 10 CFU/100 cm^2). For comparison, up to 4% the untreated samples from hind leg and at least 36% of the samples from neck were observed to contain at least 10 CFU/100 cm^2. The apparent differences in the effects of PAA and ClO_2 at different sites agree well with the data obtained from the trials using inoculated carcases (*see* above). This reinforces the idea that some parts of the carcases were not exposed properly to spray during chilling and that a proper coverage of carcases with an oxidant solution is required to achieve the highest antimicrobial effects at all sites on the carcases.
- The MPN data of the present trials also revealed that ClO_2 application generally produced greater antimicrobial effects than those of PAA application at all test sites (Figures 6-10). This differs from the trials using inoculated carcases in which PAA was more effective against *E. coli* than ClO_2. The inconsistent observations between trials using uninoculated carcases and carcases inoculated with *E. coli* are consistent with the fact that different concentrations of PAA and ClO_2 were used in those trials. The concentration of PAA (at 120 ppm) and ClO_2 (at 80 ppm) tested in the trials using uninoculated carcases were different to the proposed concentration (*i.e.,* PAA at 200 ppm and ClO_2 at 50 ppm). This could be due to the fact that the test oxidants did not mix properly with water in the bulk tank although the process of chemical dosing was overseen by a manufacturer's representative in one of the trials. Further trials using the correct concentration of PAA and ClO_2 are, therefore, required to further resolve the apparently inconsistent observations between the two types of trials.

Future work and considerations

- As already described above, there are a number of issues that should be addressed in order to maximise the effectiveness of the test application as an antimicrobial intervention. These include improper coverage of carcases with an oxidant solution during spray chilling and incorrect concentration of the test oxidant in the bulk tank. Development/implementation of an effective delivery and dosing systems to achieve better coverage and the correct concentration should be considered, respectively.
- To facilitate commercial adoption of the test application as an antimicrobial intervention, a feasibility study should be conducted. This would involve assessment of both advantages and

disadvantages of each intervention, including evaluation of its cost *vs.* antimicrobial benefit, Workplace Health and Safety considerations, etc.

- It is also important to understand the impact of the test application on beef quality before commercial adoption. This would involve evaluation of both shelf life and sensory characteristics of vacuum-packed beef.

Conclusion

- The data from these six trials confirms that application of an oxidant (*i.e.,* either PAA or ClO_2) during spray chilling is very effective in reducing *E. coli* prevalence and numbers on carcases.
- The test application can be implemented commercially as an effective antimicrobial intervention for beef carcases.
- However, future work is still required to ensure that the effectiveness of the test application is maximised (*i.e.,* addressing the issues with the delivery and dosing systems), assess the cost-benefit and evaluate its impact on beef quality prior to its commercialisation and industry adoption.