final report

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1 Introduction

Recent introduction of PCR testing for *E. coli* O157 and expansion of testing to include an additional six Shiga-toxigenic *E. coli* (STEC) has meant that the Australian industry is faced with increased detections of these pathogens. This is due to the larger number of pathogens of concern as well as the increased sensitivity of the diagnostic tests, despite meat processing practices remaining largely unchanged. Further, existing process control measures, including Product Hygiene Index and *E. coli* and Salmonella Monitoring (ESAM), are focussed on general processing hygiene and have been shown to have low ability to predict likely pathogen detections. That is, the presence of STEC confirmation has occurred despite generic *E. coli* not being detected on carcases, or at very low levels, and little or no visual carcase contamination.

Consequently, a processor was interested to determine whether or not expanded microbiological process control measuring, similar to United States Department of Agriculture (USDA) Agircultural Research Service (ARS) and IEH Harvest Monitoring, would assist the company in identifying areas to affect process control improvements and reduce the risk of STEC detections impacting operations.

2 Aims

To establish process control microbiological baselines at company A beef processing plants at Establishment A, Establishment B and Establishment C. In particular, the following questions were of interest.

1. Which (of the) specific carcase sites or aspects of the process lead to higher microbiological contamination on average?
2. Are there significant differences in average microbial concentrations between different days/shifts?
3. Are there significant differences in average microbial concentrations between left and right carcase halves?
4. Do plants differ significantly in average microbial concentrations?
5. Are there significant differences in average microbial concentrations between samples collected immediately after hide removal and samples collected after final trimming?
6. Is it possible to determine if there is any correlation between (logistical) factors such as the cleanliness of the cattle pre-slaughter, cattle breed, the chain speed on the kill floor, number of workers and supervisors, etc. and the microbiological results?
7. Are Statistical Process Control (SPC) charts useful for monitoring process control?

3 Methods

3.1 Sample Collection

At each of the three plants, six consecutive carcase swab samples were collected each shift – two shifts at Establishment A and one at Establishment B and Establishment C – for a period of ten days. Sampling
was undertaken at Establishment A in November 2012, at Establishment B in November and December 2012 and at Establishment C in December 2012.

Samples were collected using Nasco whirlpak sponges hydrated with approximately 10mL of Buffered Peptone Water (BPW).

Six consecutive carcases from the same group of animals were selected. Separate samples were collected from the flank, brisk and rump areas on one carcase side shortly after hide removal, and from the opposite side of the same carcase after trimming, near the grader. A single sterile glove was worn for collecting the samples from the six consecutive carcases before being changed for the next six samples.

For logistical reasons, all carcases had the same carcase side swabbed after hide removal during the first week and the other side was swabbed in the second week. It was acknowledged that this would lead to confounding of carcase sides and weeks.

The Rump, Belly and Forequarter swab sites focussed on hide removal cutting lines. The before trimming Rump samples were collected on the legging stand just after both legs were hooked up at all sites. The before trimming Belly samples were collected on a fixed stand just before the brisket saw at Establishment A and on the viscera table immediately after the brisket saw at Establishment C and
Establishment B. The before trimming Forequarter samples were collected just before the brisket saw at all plants.

The after trimming Rump sample was collected from a rise and fall stand immediately after the grader at Establishment C and Establishment B, and after the grader and feather boners at Establishment A.

The Belly and Forequarter after trimming samples were collected after the grader and feather boners at Establishment A, immediately after the grader at Establishment C and immediately before the grader at
Establishment B.

After sample collection, BPW was added to each sample to make a total volume of 25mL. Samples were transported chilled to the laboratory after completion of all sampling for a shift had been completeEstablishment C.

There were transport issues with samples from Establishment C and Establishment B where some samples arrived warm. These were discarded and replacement samples were taken on another day.

3.1.1 Sample Area

It is important to note that the swabbed sample area differed between sample sites, carcases, plants and sample collectors, of which there were ten in total. Consequently, results were reported as cfu/sample. Sample areas were approximately 400 – 700cm² at the Rump, 400 – 600cm² at the Belly and 300 – 600cm² at the Forequarter. The large variability in the sample area restricts the comparison between sample sites, different carcases and different plants, in addition to ESAM results which are reported as cfu/cm². It should be noted, that the variability in sampling area alone could impact the log10 cfu/sample results by up to 0.3 log10 cfu/sample. For the purposes of this report and to aid comparisons, the assumption is made that all swabbed areas in this trial are the same size, even though the sampling method differs in reality.

ESAM samples were taken from "standard" swabbed areas of 300cm². To compare ESAM results to those of this trial which were presented as cfu/sample, ESAM results were multipled by 300 before data analysis to give results as cfu per sample.

3.2 Microbiological Testing

Sample temperatures were recorded on arrival at the laboratory. Samples were refrigerated overnight and tested the day after sample collection. Quantitative tests for TVC as per AOAC 990.12 method (Aerobic Plate Count in Foods, Dry Rehydratable Film (Petrifilm Aerobic Plate Count) Method) and for *E. coli* as per AOAC 991.14 (Coliforms and Escherichia coli Counts in Foods, Dry Rehydratable Film (Petrifilm Count Plate) Methods) were performed on the samples as received by the lab.

For qualitative *E. coli* enrichment, approximately 50mL of BPW was added to the samples after aliquots for qualitative testing had been removed and the samples were incubated at 35 ± 1ºC for 3 – 4 hours. The *E. coli* test was then performed on the samples following AOAC 991.14. The samples were plated neat; no dilutions were plated. The result was reported as *E. coli* Detected or Not Detected in the sample.

3.3 Statistical Analyses

All results for TVC and *E. coli* were log10 transformed and these log-transformed results were used as response variables for regression models and analysis of variance (ANOVA) fitted to the concentration or detection data. The main explanatory variables of interest were process stage (Before/After trimming), sample sites (Rump/Belly/Forequarter) and carcase sides (Left/Right). After an exploratory data analysis of all three plants, the counts of coliforms and *E. coli* were very similar before and after trimming and so almost all the coliforms detected were *E. coli*. As such, coliforms do not give additional information to the *E. coli* results and to prevent duplication of the analysis of *E. coli*, the analysis of coliforms is excluded from this report.

All analyses were performed with the statistical software R (R Core Team, 2012) and all R output for the models is included in Appendix C. For the following models, the significance of the treatment effects was tested at the 5% significance level and the highest-order non-significant effect was removed, based on P-value, and the model refitted. These steps were repeated until all remaining effects were significant – this was the process of model selection.

3.3.1 TVC

In the summary of carcase TVC levels, the microbial concentrations before and after trimming were compared by taking the difference between After and Before log10 concentrations for each individual carcase.

The standard deviation within shifts (Within SD) was calculated from carcase results after shift differences were eliminated. The between-shift standard deviation (Between SD) was calculated as the standard deviation of the shift means.

For each of the plants, a statistical test was completed using one-way analysis of variance (ANOVA) to assess whether shifts differed significantly on average in log10 TVC.

A linear mixed effects model was fitted separately for each plant to determine differences in the microbial concentration:

- between carcase samples before and after trimming
- between carcase samples taken at the Rump, Belly and Forequarter
- between left and right sides of the carcases

and the interactions between these treatments; day-to-day variation was included as a random effect.

P-values were calculated using the likelihood ratio test or Markov Chain Monte Carlo simulation.

Statistical Process Control Charts

Statistical process control analysis/charts are used to establish process control baselines and monitor the process on an ongoing basis for process control. One example is *x* and R charts which are fitted to data grouped into observations per shift and day combination. *x* charts display the mean on a shift-by-shift basis and show the level of variability between shifts. R charts show the range of values within each group of six carcases, that is the carcase-to-carcase variability within a shift.

"Training" or lead-in data is used to assess the process control and establish average patterns which are represented by the limits in the chart. Out-of-control patterns are indicated by points outside of the control limits and for the training data, overall patterns are removed (one-by-one) until a stable process is achieved.

It is important that reasons for outliers or out-of-control trends are investigated (as far as possible) to avoid further occurrences if detrimental or to replicate good outcomes.

3.3.2 *E. coli*

At Establishment A, the same full mixed effects model as for TVC, incorporating before/after trimming,
sample site, carcase side and day-to-day variation, was also applied to log10 *E. coli* levels.
At Establishment B and Establishment C, a survival model was fitted to only those samples which showed *E. coli* growth and were detected as positive after enrichment. This model allowed for the censoring introduced by counts falling below the lower limit of detection (Lorimer & Kiermeier 2007). The *E. coli* concentrations below the limit of detection (<25 cfu/sample) were replaced with the lower limit. Trimming, sample site and carcase side were included in this model as fixed effects

explanatory variables.
To assess the influence of trimming, sample site and carcase side on the detection of *E. coli* in enriched samples, a logistic regression of the *E. coli* detection was applied.

3.3.3 Comparison of Plants

In addition to statistically analysing the three plants of Establishment A, Establishment B and Establishment C
individually, all plant data was combined to make comparisons of levels of TVC and *E. coli* detection on enriched samples between the different plants.

A model similar to the full model applied to TVC, was fitted to determine differences in the TVC levels:

- between Establishment A, Establishment B and Establishment C
- between carcase samples before and after trimming
- between carcase samples taken at the Rump, Belly and Forequarter

and the interactions between these treatments. This model also included the random day effect.

The same logistic model as for the enriched samples was used to compare plants' concentrations of *E. coli*.

4 Results

4.1 Summary of results of samples taken after hide removal and before trimming

- There was variability between sites and between sides, but the differences were not consistent between the plants.
- At Establishment A the left forequarter sample results were significantly higher, possibly related to the Halal cut causing increased bleeding over this site. The Halal cut is not done at the other plants.
- At Establishment C the forequarter sample results were lower than the rump and belly.
- Establishment C had the highest TVC and lowest *E. coli*, Establishment B the lowest TVC and Establishment A the highest *E. coli*
- *E. coli* prevalence was close to 100% for all sites at all plants by the qualitative method.

4.2 Summary of results of samples taken after trimming

- Significantly lower than before trim results.
- TVC results were 1.1, 0.4 and 0.6 $\log_{10}$cfu/sample lower at Establishment A, Establishment B and Establishment C respectively.
- *E. coli* results were 0.6, 0.3 and 0.3 $\log_{10}$cfu/sample lower at Establishment A, Establishment B and Establishment C respectively.

- Little difference between the three sites or between sides at all plants
- Establishment C had some variation with the forequarter slightly better and the belly slightly worse.
- There was variation between plants. The average TVC results at were 3.2, 3.1, and 4.0 $\log_{10}$ cfu/sample at Establishment A, Establishment B and Establishment C respectively.
- *E. coli* prevalence was approximately 80% for all sites at all plants by the qualitative method and 30% by the quantitative method. This indicates that the quantitative method underestimates the prevalence of *E. coli* as it is often present below the limit of detection.
- Higher before trim results did not correlate well with higher after trim results for specific carcases. There was slight correlation at Establishment B. Therefore before trim results are not a good indicator of after trim results and matching of sides is not useful when collecting samples.

"Statistical process control charts were limited in their usefulness. The presence of a large number of outliers resulted in the conclusion that it is not feasible to say that within-group and between-group variability is consistent or remains stable over time. As such, the statistical process control charts have limited value for monitoring statistical process control"