AUSTRALIAN MEAT PROCESSOR CORPORATION

Assessing the effectiveness of a carcase hot water decontamination cabinet in small stock processing

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1.0 Executive Summary

The United States Department of Agriculture (USDA) Food Safety and Inspection Service applies a zero tolerance policy for contamination on meat by faeces, ingesta and milk. Recent history with rejections of consignments from findings of zero tolerance on sheep and goat carcasses and six-way cut pieces at port of entry inspection in the US has highlighted the challenge in consistently meeting this policy standard in these categories of products.

The key objective of this project was the trialing of a hot water spray wash cabinet at a single slaughtering establishment to determine its effectiveness in the removal of visual defects from the surface of sheep and goat carcasses under normal commercial operating conditions.

In addition to assessing the effectiveness of the cabinet in removing visual defects, a microbiological study was commissioned in association with the trial to assess bacteriological impacts from the hot water on the sheep and goat carcasses.

The operational parameters of the hot water spray wash cabinet under which data was collected for analysis were:

- Hot water temperature at cabinet console 93 – 94°C with temperature at times exceeding 95°C. Hot water at the surface of the carcase 60 – 65°C with temperature at times exceeding 65°C
- Carcase contact period with the hot water spray not less than three seconds. Water pressure 16 bar through the spray heads
- Chain speed 7 – 7.5 carcasses/minute
- Single carcase in the cabinet at one time
- All stock processed during this data collection period were of good quality with minimum levels of dust in the pelts; no observable scouring; no wet and dirty pelts and legs; and minor seed infestation.

The trial found that the cabinet to be generally effective in removing loose fibre (wool or hair) and dust, though not all (and notably hair from goats). The cabinet was generally effective in removing loose faecal pellets. However, embedded and adhered faecal material, spillage and frank stains/smears remained or were only partially removed with visual evidence of the original contamination clearly obvious.

The trial also found that the hot water treatment had no negative impacts on carcase microbial counts. Reductions in bacterial load for coliform and *E. coli* were achieved with the effects of the hot water spray wash cabinet and overnight air chilling. However, the combination of these two treatments is not an elimination step for bacterial contamination.

It is recognised that further design of the hot water spray wash cabinet may be necessary for adoption at higher speed establishments. The hot water spray wash cabinet does not replace the need of present procedures for visual examinations of carcasses and piece meats to confirm compliance with the zero tolerance policy for contamination by faeces, ingesta and milk.

1.0 Background

The United States Department of Agriculture (USDA) Food Safety and Inspection Service applies a zero tolerance policy for contamination on meat by faeces, ingesta and milk. Recent history with rejections of consignments from findings of zero tolerance on sheep and goat carcasses and six-way cut pieces at port of entry inspection in the US has highlighted the challenge in consistently meeting this policy standard for these categories of products.

A principal concern with the delivery of the zero tolerance policy is that it amounts in practice to intolerance of any visible contamination on carcasses because of the difficulty in distinguishing between specks of different materials. Accordingly, an enormous effort is expended on activities aimed at ensuring no specks from any form of contamination can be observed on carcasses leaving the slaughter floor in order to remove ambiguity in interpretation.

Zero tolerance defects relate to the visual identification of faeces, ingesta and milk on the carcasses and meat. The fundamental assumption underlying this policy is that enteric pathogens found on meat are derived from faecal matter or milk that is deposited on the carcase during slaughter and dressing operations. The ultimate source of these types of contamination is either through spillage from the gastrointestinal tract or udder or through the transfer of the contamination from the hide or fleece during dressing of the carcase.

It is well established that most of the bacteria on carcasses are deposited on the meat during skinning operations. It is generally accepted that meat will be microbiologically safe if the transfer of contamination from hide to carcase is minimised, spillage of gut and udder contents mostly avoided and any visible contamination that is present on the carcase removed by trimming or vacuum cleaning.

Hot water treatments have been shown to remove faecal material and to improve the visual appearance of the tissue in line with the USDA's zero tolerance policy. Hot water can be applied during slaughter as a whole carcase wash or to specific areas of the carcase. Application can be by spray (high or low pressure, manual or automatic), by deluge in a cascade or by immersion.

Hot water as an intervention step has been extensively researched. Sheep and beef sides are treated for up to 15 seconds with 75°C -95°C water, with reductions of up to three log of pathogenic and spoilage bacteria being reported.

Hot water sprays may not achieve the desired temperatures at the contact surface but may remove visible contamination. Low pressure sprays would provide higher tissue temperatures than high pressure as it allows a longer contact time, but high pressure is more able to remove visible contamination.

The position of the intervention on the chain is important. Researchers have shown that washing carcasses immediately after defleecing may inhibit further attachment of bacteria later in the process. Hot water applied before any other washing gives a mean reduction in total count of 1.3 log compared with a mean reduction of 0.8 log if the hot water intervention is applied after a cold water wash.

2.0 Project objectives

The key objective of this project was to determine the effectiveness of a hot water spray wash cabinet at removing visual defects from the surface of sheep and goat carcasses under normal commercial operating conditions at a single slaughtering establishment.

A microbiological study was commissioned in association with a trial to assess bacteriological impacts from the hot water on the sheep and goat carcasses.

3.0 Materials and methods

A hot water spray wash cabinet was developed at a single slaughtering establishment to trial the effectiveness of hot water in reducing visual contamination on the surface of sheep carcasses as well as skin-off and skin-on goat carcasses.

By achieving reductions in visual carcase contamination, the cabinet could assist in providing processors with an additional risk mitigation measure for meeting USDA market access standards for zero tolerance from faecal and milk contamination.

Visual contaminants included within the scope of the trial are zero tolerance defects (faeces, ingesta and milk) as well as other defects, such as fibre and hide dust.

The intent for the trial was that hot water be delivered across the entire surface of the carcase through a series of sprays within an enclosed in-line cabinet immediately following carcase evisceration and prior to carcase post mortem inspection. The cabinet was custom designed for this exercise.

It was intended the trial methodology assess the effectiveness of the cabinet operation to determine whether the contamination in question has been removed in full, only partly removed (evidence of the contamination remains), or whether the contamination in part or full has been relocated elsewhere on the carcase.

It is recognised that the hot water spraying will have little or no effect on certain categories of carcase defect including pathology (parasitism, local infections, arthritis, neoplasms, etc.), grass seed infestation and injury.

Given the location of the cabinet on the slaughter line, it was also recognised that carcasses with peracute pathologic conditions such as fever, pyaemia and serious suppurating infections would be condemned prior to those carcasses entering the decontamination cabinet to minimise the potential for cross contamination from contact with the cabinet and related fixtures. Accordingly, a trained quality control officer was positioned to inspect every carcase before it entered the cabinet for the purpose of ensuring carcasses with peracute conditions and carcasses with obvious zero tolerance defects were not subject to washing through the cabinet in line with regulatory expectations.

A protocol for the microbiological assessment of carcasses passing through the cabinet was developed based upon previous methods used in studies designed to assess the effectiveness of hot water decontamination on beef carcasses.

In order to investigate the decontamination effects of the hot water cabinet on sheep, skin-off goat, and skin-on goat carcases, two studies were conducted – a challenge study and an incidental contamination study.

Image 1: Entry doors to enclosed spray wash cabinet.
Hot water treatment is turned off at the console for a heavily contaminated carcase. Water temperature is monitored at the console by digital display. The temperature is typically between 93-94°C. The two doors open to permit the entry of a carcase.

Image 2: Exit doors following hot water treatment.
The spray wash cabinet is designed for a single carcase at a time to minimise cross contamination. The chain speed is 7.5 carcasses/minute.

Image 3: Sprays deliver hot water between 60-65°C at the surface of the carcass for three seconds at 16 bar pressure.

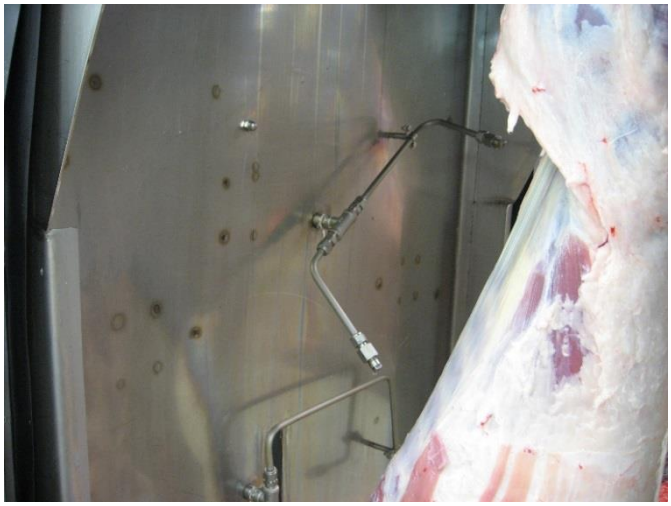

Image 4: Hot water sprays turn on when cabinet doors close to assist in controlling hot water and aerosols.

There is minimal contact between the carcase and cabinet other than for occasional contact with shank tips and cabinet doors, particularly with larger carcasses.

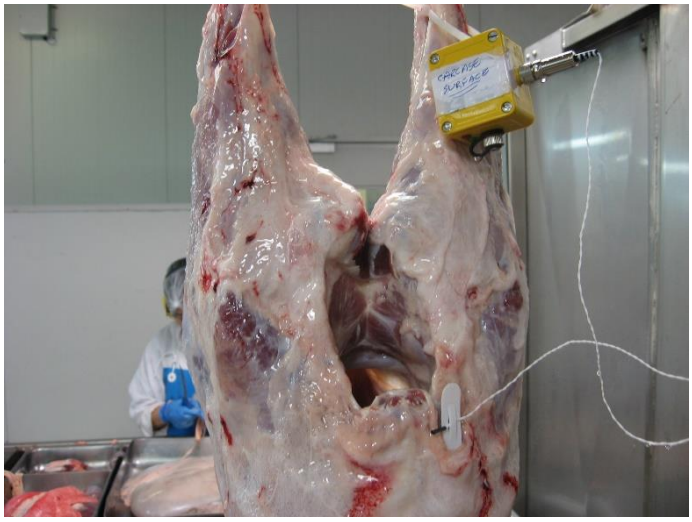

Image 5: Determining temperature of hot water at the surface of the carcase.

Engineering during the project resulted in temperature increases on the carcase surface from 45–50°C to 60 – 65°C in the lead and trail flanks, mid back, lead and trail shoulder and brisket. Final data collection was at the higher temperature range.

3.1 Challenge study

An *E.coli* culture with five mixed (inert) strains was applied to each of the five sampling sites (3 ESAM sites plus the hind hock and the shoulder) on the carcasses. Sponge samples were collected as per the ESAM swabbing procedure, prior to and after hot water decontamination, and after overnight chilling. Sponge samples from the five sites on each carcase were aggregated in three bags and dispatched to a NATA accredited laboratory for testing for *E.coli* and coliforms.

The trial was conducted on two different occasions. Six (6) carcasses of two categories (sheep and skin-off goat) were sampled for testing on the first occasion, while eight carcasses of three categories (sheep, skin-off goat and skin-on goat) were sampled for testing on the second occasion.

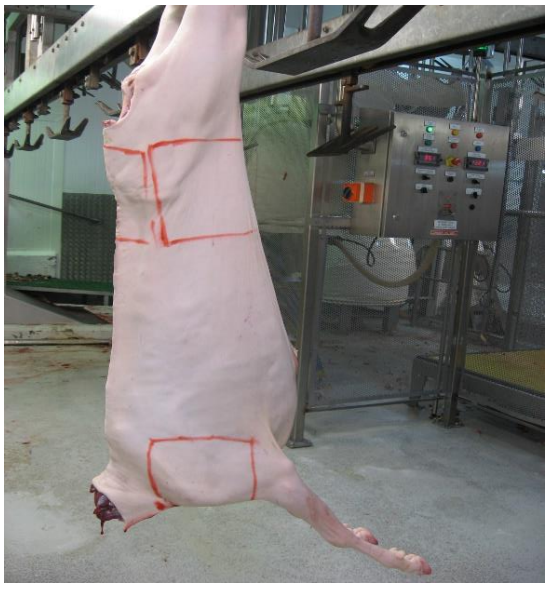

Image 6: Sampling sites for skin-on goat carcase.

3.2 Incidental contamination study

The same treatment and sampling procedures were used for the challenge study, except that no *E.coli* culture was deliberately applied to the carcasses. Sponge samples were sent to a NATA accredited laboratory for testing for SPC, *E.coli* and Coliforms. The trial was conducted on two different occasions. Ten (10) carcasses of two categories (sheep and skin-off goat) were sampled for testing on the first occasion, 95 carcasses of four categories (26 sheep, 25 lambs, 20 skin-on goats, 24 skin-off goats) were sampled for testing on the second occasion.

A copy of the microbiological testing protocol submitted to the Department of Agriculture is included in Attachment 1.

In the prelude to the trial, carcase hygiene from the examination of group of sheep carcasses in chillers was found excellent, thereby indicating the effectiveness of the range of measures applied during primary processing in either preventing or otherwise removing contamination from carcasses prior to their removal from the slaughter floor. This observation was reflected in the detailed assessment of carcasses following their breakdown in the boning room.

The project commenced in January 2014 with final data collections made in early in February 2015.

Image 7: Swabbing skin-on goat carcase.

Image 8. Example of typical sheep carcase during data collection.

4.0 Results and discussion

A key aspect of the project was the range of adjustments made in the engineering of the spray wash cabinet in light of experience with its operation. A series of modifications aimed at improving the performance of the cabinet were made throughout the period of the project to attain the operational parameters used in the final data collection.

The operational parameters of the hot water spray wash cabinet under which data was collected for analysis were:

- Hot water temperature at cabinet console 93 – 94°C with temperature at times exceeding 95°C. Hot water at the surface of the carcase 60 – 65°C with temperature at times exceeding 65°C
- Contact period not less than three seconds. Water pressure 16 bar through the spray heads
- Chain speed 7 – 7.5 carcasses/minute
- All stock processed during this data collection period were of good quality with minimum levels of dust in the pelts; no observable scouring; no wet and dirty pelts and legs; and minor seed infestation.

4.1 Visual defects

Data relating to the assessment of visual defects on carcasses before and after the hot water spray wash cabinet are included in Attachment 2. Diagrammatic representations of the data are presented below. The data is derived from carcasses processed under normal conditions at the trail establishment.

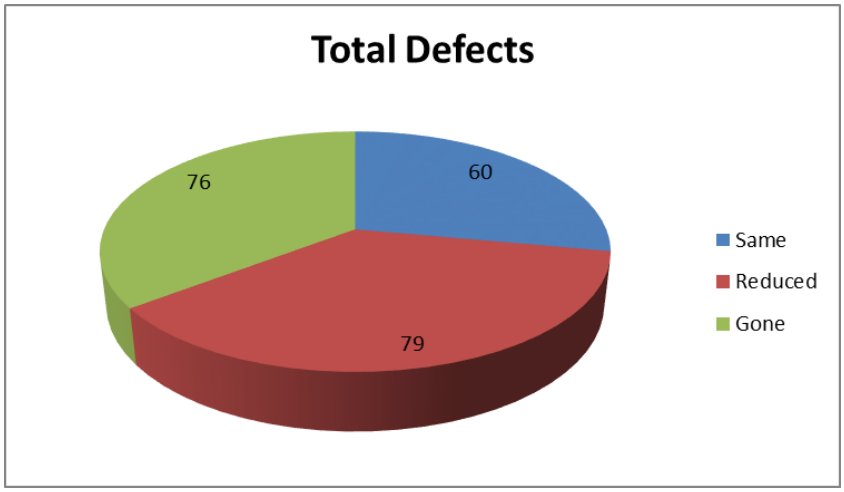

Diagram 1: Presentation of data collected mid-January 2015 to early February 2015. Total defects from sheep: 35% of total defects removed. 65% remain entirely or in a reduced form.

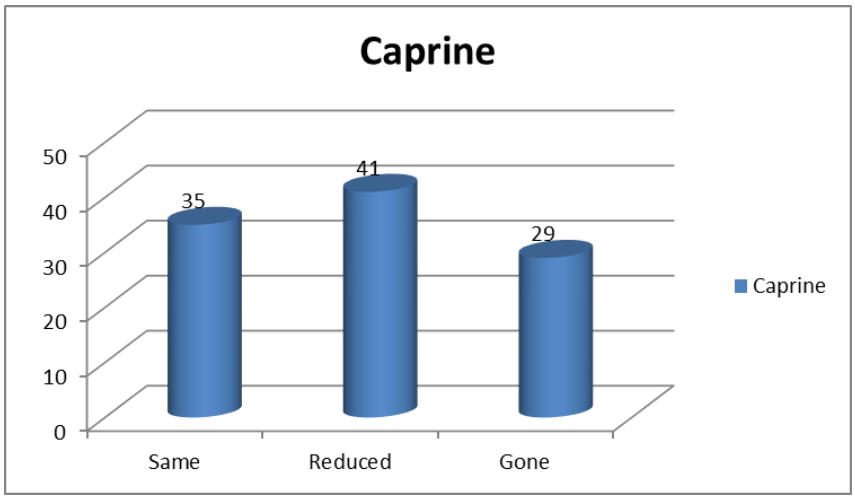

Diagram 2: For goats, 27% of total defects are removed. 73% of defects remain entirely or in reduced form. The most common defect is hair. 33% of defects have not been removed or reduced by the hot water spray wash cabinet.

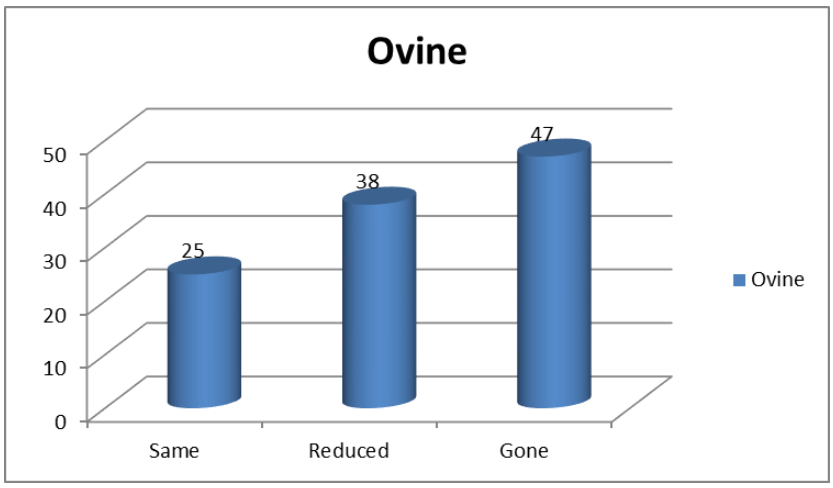

Diagram 3: For sheep, 43% of total defects are removed. 23% of defects have not been removed or reduced by the hot water spray cabinet.

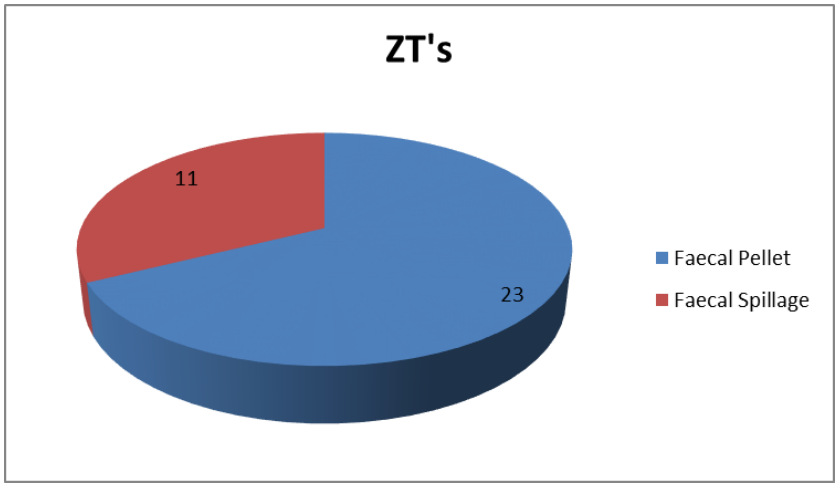

Diagram 4: 67% of total zero tolerance findings from sheep and goat carcasses are faecal pellets. 33% are in the form of smears, stains and frank spillage.

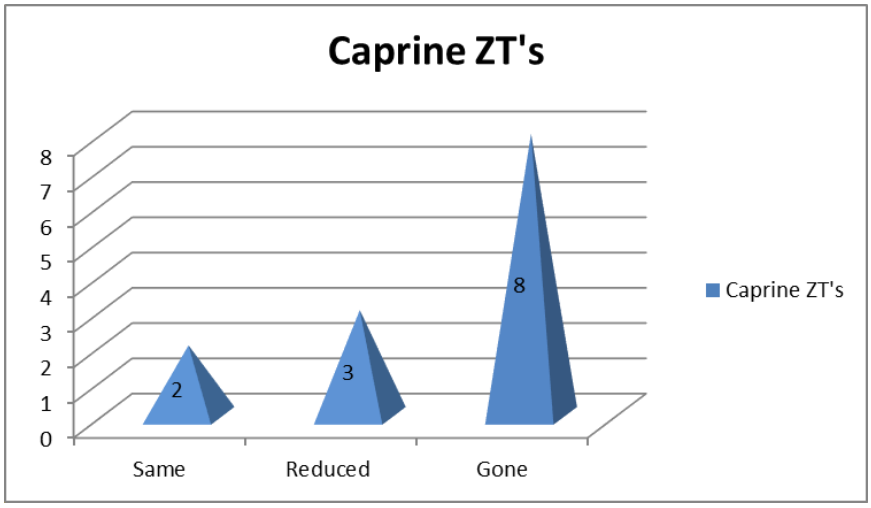

Diagram 5: 62% of zero tolerance defects on goat carcasses are removed entirely by the hot water spray wash cabinet. These are the faecal pellets sitting loosely on the surface of carcasses.

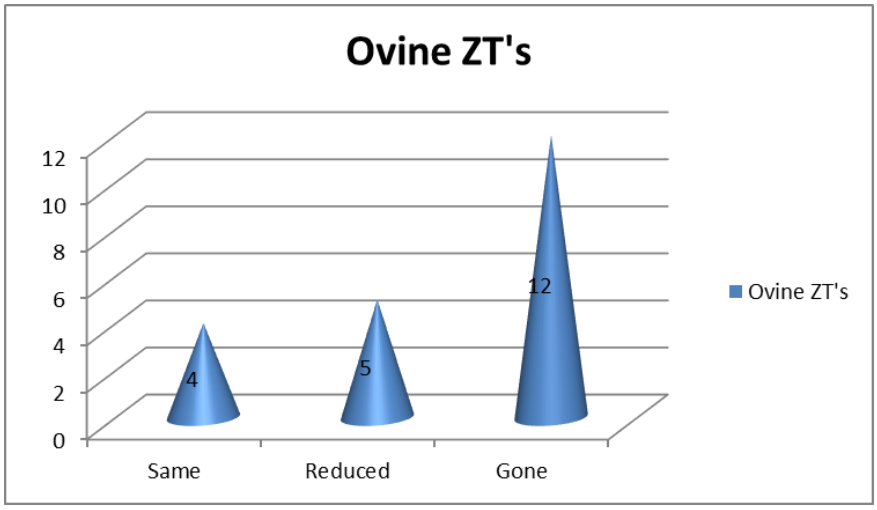

Diagram 6: 57% of zero tolerance defects on sheep carcasses are removed entirely by the hot water spray wash cabinet. As with goats, these are the faecal pellets sitting loosely on the surface of sheep carcasses.

4.2 Microbiological status

The data sets from the microbiological component of this project are included in Attachment 3. It is also important to note that the sample numbers involved in these studies are relatively small and that the specific attribution of effects to the hot water treatment needs careful interpretation when the outcome may largely reflect the effects of the chilling of carcasses overnight.

This data is presented in Tables 1 and 2.

4.2.1 Challenge study

Table 1: Results (mean counts) expressed as log/cm² reductions in microbiological counts.

	INOCULATED; NO HOT WATER SPRAY, OVERNIGHT CHILL		INOCULATED; HOT WATER SPRAY, OVERNIGHT CHILL	
	Coliform	*E.Coli*	Coliform	*E.Coli*
Mutton	2.3	2.2	2.5	2.6
Skin on goat	-0.2	-0.1	3.0	3.1
Skin off goat	0.0	0.0	1.7	1.7
Total average	0.7	0.7	2.4	2.5

The swabbing of carcasses under the challenge study to determine reductions in microbiological counts was made after overnight chilling. Accordingly, count reductions in the two groupings reflect the effect of chilling. For the group receiving the hot water spray, reductions in microbiological counts reflect the combined effect of the hot water and subsequent chilling.

4.2.2 Incidental contamination study

Table 2: Results (mean counts) expressed as log/cm² reductions in microbiological counts.

	DIFFERENCE IN COUNT BETWEEN HOT WATER SPRAY AND NO HOT WATER SPRAY CARCASSES		
	Total viable count	Coliform	*E.Coli*
Mutton	0.4	0.2	0.3
Lamb	0.1	0.0	0.0
Skin on goat	0.0	0.3	0.3
Skin off goat	0.2	0.1	0.1

The swabbing of carcasses under the incidental contamination study to determine reductions in microbiological counts was made after overnight chilling. Accordingly, the difference in count reductions between the two groupings also reflect the effect of chilling.

5.0 Conclusions and recommendations

5.1 Visual defects

There are a number of general outcomes from the visual assessment of defects on sheep and goat carcasses before and after the hot water spray wash cabinet:

- The cabinet is generally effective in removing loose fibre (wool or hair) and dust, though not all (and notably hair from goats). Matter firmly attached to fascia was only partially or not removed
- The cabinet was generally effective in removing loose faecal pellets, imbedded faecal material, spillage and frank stains/smears remained or were only partially removed with evidence of the original contamination clearly obvious
- Carcasses (particularly sheep) had a glisten following their hot water treatment which improved the appearance of the carcase and may have the effect of improving the ease of detection of remaining defects on the carcase by subsequent trimming operations following carcase inspection.

5.2 Microbiological status

There are a number of general outcomes from the microbiological assessment of sheep and goat carcasses:

- the hot water treatment had no negative impacts on carcase microbial counts.
- for inoculated carcasses from the challenge study, significant reductions in bacterial load for coliform and *E. coli* were achieved with the combined effects of the hot water spray wash cabinet and chilling. However, the combination treatment of hot water and chilling is not an elimination step for bacterial contaminants.
- the most pronounced reduction in the challenge study was observed in skin-on goats where a three log reductions in coliform and *E. coli* were recorded. However, under normal conditions of operation these reductions were less pronounced (0.3 log reduction), perhaps reflecting lower initial bacterial counts during actual processing.

5.3 Recommendations

The trial was undertaken at a single slaughtering establishment with a modest chain speed. It is recommended that trailing also be considered at high speed establishments as part of any broader industry adoption. It is envisaged that cabinet design and location of cabinet may require further consideration within a high speed context.

The hot water wash cabinet does provide an opportunity to review the benefit of other washing and vacuum operations within the dressing operations. It is recommended that these approaches be carefully considered along with any subsequent carcase inspection steps to ensure zero tolerance defects have been effectively removed.

Acknowledgment

The assistance of Jean Tabra, Dave Elkington, Neville Hensel, Joe Liu and Ian Eustace throughout this project is gratefully acknowledged.

6.0 References

Food Science Australia Meat Technology Update 03/2 Pathogen reduction interventions for carcasses April 2003.

Food Science Australia Meat Industry Services Hot Water Rinse Updated June 2006.

Food Science Australia Meat Technology Update 05/10 Sources of contamination on beef carcasses during dressing November 2010.

7.0 Appendices

7.1 Appendix 1 - Final protocol for submission to the Department of Agriculture: Approval to undertake microbiological assessment of small stock intervention system

Purpose of submission

To describe in detail the procedure proposed to undertake a challenge study with *E. coli* on the small stock slaughter floor and in small stock chillers at a small stock abattoir to enable submission to the Department of Agriculture for consideration of approval.

Purpose of microbiological assessment

To provide the Australian small stock processing industry with microbiological and carcase temperature data for the validation of the hot water wash cabinet system installed in 2013 and assessed for performance on visible contaminants to June 2014.

Background

A hot water wash cabinet has been developed as an aid to reduce visual and microbiological contaminants across the surface of small stock carcasses. All carcasses are assessed for visible contamination prior to entry into the cabinet. Carcasses having visible zero tolerance defects are not subject to washing through the cabinet.

In achieving reductions in visual carcase contamination, the cabinet should assist by providing an additional mitigation measure within the tool kit of processors for meeting United States food safety standards for zero tolerance at port of entry inspection.

Assessments undertaken during February and April led to modifications to the cabinet and an opinion was reached in June 2014 that the engineering of the cabinet had largely reached an end point where operational parameters were delivering hot water in the cabinet at a temperature of 90°C (±2°C) at 20 bar pressure for a three second contact period.

AMPC now wishes to proceed to the proposed microbiological assessment of the investigation by temperature measurements on test carcasses passing through the cabinet, by undertaking a microbiological challenge study, and by a sampling program of incidental bacterial contamination.

Description of the hot water cabinet

The carcase wash cabinet is located immediately following evisceration and prior to carcase examination. The stainless steel cabinet has a set of sprays on each side which spray the carcasses with high temperature water as they pass through. Water is delivered constantly from spray heads at a temperature of 90°C (± 2°C). Operation of the sprays is controlled by sensors. Water pressure to the sprays is 20 bar. The cabinet is fitted with sliding doors at entry and exit and an exhaust to remove vapour. Recent engineering enhancements include the stabilising carcasses during passage through the cabinet, additional sprays and alignment of the sprays to provide improved coverage.

Principle of study

In-plant validation studies will be undertaken by:

1. Temperature measurement on test carcasses passing through the wash cabinet.
2. Applying to test carcasses that have been excluded from commerce, one or more *E. coli* strains that contain no known virulence markers for pathogenic *E. coli* (i.e. are considered to be non-pathogenic). Reference will be made below to previous in-plant challenge studies in which these strains have been used.
3. A sampling program over periods after implementation of the intervention to compare incidental bacterial contamination of carcases before and after their treatment in the cabinet.

Materials and methods

Temperature Measurement

During a rest break, a fast-response sensor connected to a Tinytag temperature logger will be attached to a carcase at one of the sites designated for the bacterial studies (see 2. below) such that the sensor is approximately 5-10 mm above the surface. The carcase will be conveyed through the cabinet. The temperatures will be logged at one second intervals. At subsequent breaks, further carcases will be similarly instrumented at the various designated sites and conveyed through the cabinet. The temperature data will provide information on the temperature reached at several locations near carcase surfaces and the duration of temperature lethal to bacteria.

NOTE: It may be possible to fit the loggers and probes during normal slaughter floor operation. If so, the necessary temperature logging can be done then.

Challenge testing with known strain of *E. coli*

Preparation of bacterial culture

Non-pathogenic *E. coli* strains (from EC1604, EC1605, EC1606, EC1607, EC1608 – strains that contain no known virulence markers for pathogenic *E. coli* are therefore considered to be non-pathogenic) will be used. These strains are used as surrogates for *E. coli* O157:H7 and have been used for challenge testing by CSIRO scientists in uncooked fermented meat products, and on beef sides at Oakey Abattoir (2000), Australia Meat Holdings, Dinmore Facility (2000-01) and at Kilcoy Pastoral Company Abattoir (2003). As a CSIRO scientist, current Department of Agriculture Principal Scientist Paul Vanderlinde led two of those studies.

A broth culture will be delivered to the abattoir by Symbio Alliance. If possible, it will be subcultured at the abattoir by Ian Eustace and grown overnight at around 37°C in buffered tryptose soya broth (BTSB). On the day of the challenge testing, 5 mL of the culture will be added to 300 mL of sterile BTSB in a sturdy screw-top plastic container to give an inoculum containing around 10 million *E. coli* per mL. The broth culture will be stored on ice until its application to the test carcases.

Application of culture to carcasses

On each of two separate days, three sheep carcasses and three skin-off goat carcasses will be clearly tagged as test carcasses. Five sites on each of the carcasses will be then marked using a meat marking crayon or permanent marker. Sites will be the three ESAM test sites on the carcasses (mid-loin, flank area and brisket), the hind hock, and the shoulder. For each of the sites on each test carcase, an area measuring 10 cm x 10 cm will be marked and subsequently painted with the bacterial suspension using a sanitised paintbrush. The test sides will be held for around 10 minutes to allow bacterial adhesion to the carcase surface prior to their treatment. A total of 12 carcasses will be tested over the two days. Once they have been sampled after the cabinet, the carcasses will be retained for chilling, segregated from other carcasses.

Collection of samples

Samples will be collected prior to and after hot water decontamination (x, y), and after overnight chilling (z). At each sampling time, areas 5 cm x 5 cm will be swabbed at each of the five sites from within the marked areas for each of the test carcasses. The swabbing procedure will be that specified for ESAM sampling. Sponges from the five sites on each carcase will be aggregated in one bag, labelled with the carcase code and held chilled until dispatched to Symbio Alliance for testing.

After the chilled carcasses have been sampled, they will be transferred to the rendering facility to be rendered. This will be done in a manner that cross-contamination of other carcasses is avoided. They will be condemned and put down the condemned chute on the processing floor.

In order to determine if any of the test bacterial culture applied to the test carcasses is simply relocated by the war sprays rather than being eliminated, at least six swabs will be taken during the sample collection from areas lower on test carcasses. Also, in order to determine if the doors and internal surfaces of the cabinet might act as vectors of cross-contamination, on two occasions swab samples will be taken from each of two carcasses that are allowed to follow the test carcasses through the cabinet without any prior hot water wash-down of the doors.

Microbiological testing

Symbio Alliance will undertake microbiological testing of all samples collected following normal protocols for ESAM samples. In determining the dilutions to be plated, based on experience with beef sides, counts on samples before treatment might be expected to be in a range 10,000 to 100,000 per cm^2, those after hot water treatment and after chilling might be expected to be in the range 100 to 5,000 per cm^2. Samples from both the incidentally contaminated carcases and the inoculated ones will be tested for total counts and for *E. coli*.

Sampling program for incidental contamination

Carcasses will be selected for sampling at intervals over 10 days of processing through the wash cabinet. It is suggested that, where possible, the carcasses be selected from those tagged as unacceptable for the US market and that sheep carcasses be the ones considered first.

The carcasses will be sampled alternately before and after the hot water treatment (after chilling rather than immediately after the cabinet) such that 'pairs' are from the same consignment lots of animals. The carcasses to be sampled after chilling will be identified and tagged on the processing floor.

Depending on the prevalence of bacterial contamination and the decontaminating effectiveness of the cabinet, at least 25 pairs of data and possibly up to 50 pairs will be required for each carcase type investigated.

Sampling will be by the ESAM sponge method. Sites will be the 3 ESAM test sites on the carcases (mid-loin, flank area and brisket), the hind hock, and the shoulder. Samples will be labelled and held chilled until dispatched to Symbio Alliance for testing.

The same procedure will be followed with lamb and bobby calf carcases and with skin-off and skin-on goats.

Personnel and abattoir resources required

The plant QA Manager will liaise with on-plant veterinary staff and abattoir personnel to facilitate the studies described above. QA and laboratory staff will be required as follows:

- Temperature measurement: one person for periods up to 30 minutes on each of six occasions
- Challenge study: two people for periods up to 60 minutes on each of four occasions to prepare three carcases by tagging them and marking test areas and to swab the test areas both before and after the test carcases have been subjected to the hot water treatment. Also one person on each of two occasions to swab the carcases after chilling. One person to dispose of the test carcases. One person to package and dispatch the samples to Symbio Alliance.
- Challenge study-test carcases: six sheep carcases and six skin-off goat carcases will be directed to rendering.
- Study of incidental contamination. One person to swab five sites on each of up to 400 carcases over a period of two months.

Test data

Test results will be analysed to indicate the average reductions in numbers immediately following the treatment and after overnight chilling. The temperature data will be presented graphically to demonstrate the temperature profile near the surface of the carcase during the carcase's passage through the cabinet.

Hazard analysis

MICROBIOLOGICAL CHALLENGE STUDY – SMALL STOCK PROCESSING FLOOR CCP DETERMINATION				
Step	**Significant hazard identified**	**Justification for decision**	**What control measures can be applied to prevent the significant hazards?**	**Is this step a CCP?**
1. Application of *E.Coli* culture	B: *E.Coli* C: None P: None	Some *E.Coli* strains pathogens	Use strains with no virulence markers Cells applied in marked areas on carcase Cells applied by experienced person Containment vessels used to contain accidental spillage	No. There are further steps in the process. Which eliminate the identified hazard.
2. Sampling of test carcasses	B: *E.Coli* C: None P: None	Some *E.Coli* strains enteric pathogens	Swab sampling to be done by experienced person Swab sponges and bags to be handled with great care	No. There are further steps in the process. Which eliminate the identified hazard.
3. Transfer of test carcasses to chiller	B: *E.Coli* C: None P: None	Some *E.Coli* strains enteric pathogens	Test carcasses to be transferred under supervision of QA staff to avoid accidental contact with test areas by other staff	No. There are further steps in the process. Which eliminate the identified hazard.
4. Holding of test carcasses in chiller	B: *E.Coli* C: None P: None	Some *E.Coli* strains enteric pathogens	Test carcase retained separate from other carcasses with retained tags clearly visible. Condemn carcasses at completion of swabbing.	Yes. This is the step in the process to eliminate the identified hazard.

Risk analysis

MICROBIOLOGICAL CHALLENGE STUDY – HAZARD ASSESSMENT TABLE SMALL STOCK PROCESSING FLOOR					
Step	**Hazard**	**Cause**	**Likelihood**	**Severity**	**Significance**
1. Application of *E.Coli* culture	B: *E.Coli* C: None P: None	Some *E.Coli* strains enteric pathogens	B	3	9
2. Sampling of test carcasses	B: *E.Coli* C: None P: None	Some *E.Coli* strains enteric pathogens	B	3	9
3. Transfer of test carcasses to chiller	B: *E.Coli* C: None P: None	Some *E.Coli* strains enteric pathogens	B	3	9
4. Holding of test carcasses in chiller	B: *E.Coli* C: None P: None	Some *E.Coli* strains enteric pathogens	B	3	9

7.2 Appendix 2: Database from the assessment of visual defects

Ovine - 15	Back	dusts-1	Gone	**Before (24)**
	Neck	wool-3	Same/ 2 Gone	
		wool cluster-1	Gone	**After**
	Neck (under)	wool-1	Gone	**8 gone**
	Flank	dusts-1	Same	**11 reduced**
		wool dusts-7	7 reduced	**5 same**
		wool smear-1	Reduced	
		wool cluster-2	Same/gone	***wool cluster <3**
	Brisket	wool-1	Gone	***wool smear < 5 cm**
	Hind hock (right)	wool-1	Same	***faecal spillage < 2cm**
		dusts-1	Reduced	
	Hind hock (left)	dusts-1	Same	**ZT=0**
	Front hock (right)	wool dusts-1	Gone	
	Leg	dusts-1	Reduced	
Ovine - 15	Shank	wool smear-2	Reduced	**Before (18)**
	Leg (Right)	wool dusts-1	Reduced	

	Neck	wool-1; wool cluster-1	Reduced; Same	**After**
	Neck (Bone)	wool-1	Same	**6 gone**
	Flank	wool smear-1	Reduced	**9 reduced**
		wool-1; wool dusts-1	Gone/Reduced	**3 same**
		grass seeds-1	Reduced	
	Back	wool dusts-1; wool-1	Reduced; Gone	***wool cluster <3**
	Hind hock (Right)	dusts-1	Gone	***wool smear < 5cm**
		grass seeds-1	Same	***faecal spillage <5cm**
	Brisket	faecal pellet-1	Gone	
		wool smear-1	Gone	**ZT = 3**
	Channel	faecal pellet-1	Gone	**Faecal pellet (2) 2Gone**
		faecal spillage-1	Reduced	Faecal spillage (1) Reduced
Ovine-15	Neck	wool-2	Same/Gone	**Before (15)**
		wool clusters-2	2 Same	
		wool dusts-1	Reduced	**After**
	Brisket	wool-1	Gone	**7 gone**
	Flank	smear-1	Reduced	**5 reduced**
		wool dusts-2	Reduced/Gone	**3 same**

		wool-1	Gone	
	Back	wool smear-1	Gone	***wool cluster <3**
	Shank	wool smear-1	Reduced	***wool smear < 5cm**
	Leg (left)	wool dusts-1	Reduced	
	Channel	wool dusts-1	Gone	
				ZT = 1
				Faecal Pellet (1) Gone
Ovine-19	Back	wool cluster-1	Gone	**Before (19)**
	Leg (Right)	faecal spillage-1	Reduced	
	Leg (Left)	faecal pellet-1	Same	**After**
	Channel	wool dusts-2	Reduced/Gone	**9 gone**
		faecal pellet-1	Gone	***6*** **reduced**
		faecal spillage-1	Reduced	**4 same**
	Flank	wool-1; wool dusts-2	Gone; 2 Reduced	
	Neck	grass seeds-1; wool-1	Same; Gone	***wool cluster <3**
	Brisket	wool dusts-2	Same/Gone	***wool smear < 5cm**

		grass seeds-1	Same	***faecal spillage <5cm**
		wool-1	Gone	
	Shoulder	wool-1	Gone	**ZT = 4**
	Hock (Right)	wool dusts-1	Reduced	**Faecal Pellet (2) Same/Gone**
	Hock (Left)	wool smear	Gone	**Faecal Spillage (2) 2 Reduced**
Ovine-17	Flank	grass seeds-2	2 Same	**Before (17)**
		wool dusts-4	Same/ 3Reduced	
		faecal spillage-1	Reduced	**After**
	Channel	wool dusts-1	Gone	**8 gone**
	Brisket	wool dusts-1	Gone	**5 reduced**
	Tail bone	faecal spillage-2	Same/Reduced	**4 same**
		faecal pellet-3	3 Gone	
	Shoulder	wool-1	Gone	***faecal spillage <5cm**
	Neck	wool-1	Gone	
	Back	faecal pellet-1	Gone	
				ZT = 7
				Faecal Pellet (4) 4 Gone
				Faecal Spillage (1) Reduced

Caprine-21	Flank	hide smear-1; hair-1	Gone/Reduced	**Before (21)**
	Neck	hair-2	2 Same	
		faecal pellet-2	Same/Gone	**After**
	Back	hair-3; dusts-1	2 Reduced/Gone; Same	**4 gone**
	Channel	dusts-1; hair-2	Reduced; Same/ Gone	**10 reduced**
	Hind hock (Left)	dusts-1	Reduced	**7 same**
	Hind hock (Right)	dusts-1	Reduced	
	Front hock (Left)	dusts-1	Gone	
	Brisket	faecal spillage-1	Reduced	***smear < 5cm**
		hair-1	Same	***faecal spillage <5cm**
	Leg	dusts-1	Gone	
		smear-1	Reduced	**ZT = 4**
	Tail bone	faecal pellet-1	Same	**Faecal Pellet (3) 2 Same/ Reduced**
				Faecal Spillage (1) Reduced
Caprine-17	Hock	hair-3	3 Reduced	**Before (17)**

	Neck	hair-3	Same/Reduced/Gone/	
		hair cluster-1	Same	**After**
	Leg	dusts-1	Gone	**4 gone**
	Flank	dusts-1	Same	**8 reduced**
		hair dusts-2	Reduced/Gone	**5 same**
		hair cluster-1	Same	
		smear-1	Reduced	***hair cluster <3**
	Brisket	hair-1	Reduced	***smear < 5cm**
	Channel	hair-1	Same	
	Back	hair-2	Reduced/Gone	
				ZT = 0
Caprine-17	Flank	smear-1	Reduced	**Before (17)**
		hair clusters-3	Same	
	Back	hair-3	2 Same/Gone	**After**
	Neck	hair-3	2 Same/Gone	**3 gone**
	Shank	faecal spillage-1	Reduced	**5 reduced**
	Hock	smear-1	Reduced	**9 same**
		hair cluster-1	Reduced	

		dusts-2	2 Same	***hair cluster <3**
	Leg	dusts-1	Same	***smear < 5cm**
		faecal pellet-1	Gone	***faecal spillage <5cm**
	Channel	hair-1	Reduced	
				ZT = 2
				Faecal Pellet (1) Gone
				Faecal Spillage (1) Reduced
Caprine-17	Neck	hair-2; hair cluster-1	2 Same; Same	**Before (17)**
	Brisket	hair-2	Same/Gone	
	Hock	dusts-2	2 Reduced	**After**
		hair-1	Reduced	**5 gone**
		hair cluster-1	Same	**7 reduced**
	Shank	smear-1	Reduced	**5 same**
		hair cluster-1	Gone	
	Flank	faecal spillage-1	Gone	***hair cluster <3**
		hair cluster-1	Gone	***smear < 5cm**
	Back	dusts-1	Reduced	***faecal spillage <5cm**
		hair-1	Gone	

	Shoulder	hair cluster-1	Reduced	**ZT = 1**
	Channel	hair-1	Reduced	
				Faecal Spillage (1) Gone
Caprine-15	Neck	hair-5;	Same/ 3 Reduced/Gone;	**Before (15)**
		hair cluster-2	Same/Gone	
	Hock	dusts-2	Same/Gone	**After**
	Brisket	hair-2	2 Reduced	**4 gone**
	Back	hair-1	Reduced	**8 reduced**
	Flank	hair-1	Reduced	**3 same**
	Channel	hair -2	Reduced/Gone	
				***hair cluster <3**
				ZT = 0
Caprine-18	Back	hair-1	Reduced	**Before (18)**
		faecal pellet-2	2 Gone	
	Tail bone	faecal pellet-1	Gone	**After**
	Shoulder	dusts-1	Gone	**9 gone**
	Flank	hair dusts-2	2 Gone	**3 reduced**
		hair-2	Same/Reduced	**6 same**

	Brisket	hair -3	2 Same/Reduced	
	Hock	hair-1	Same	***hair cluster <3**
	Neck	faecal pellet-3	3 Gone	
		hair cluster-1	Same	
		hair-1	Same	
				ZT = 6
				Faecal Pellet (6) 6 Gone
Ovine-17	Shoulder	faecal pellet-1	Gone	**Before (17)**
	Flank	wool dusts-2	Reduced/Gone	
		faecal pellet-1	Same	**After**
		wool-1	Reduced	**9 gone**
		grass seeds-2	2 Same	**2 reduced**
		dusts-2	Same/Reduced	**6 same**
	Neck	faecal pellet-1	Gone	
		wool-1	Gone	***wool cluster <3**
		wool dusts-1	Gone	***smear < 5cm**
		wool clusters-1	Same	***faecal spillage <5cm**
	Tail bone	faecal spillage-1	Gone	

		faecal pellet-1	Same	**ZT = 6**
	Hock	smear-1	Gone	**Faecal Pellet (4) 2 Same/ 2 Gone**
	Channel	faecal spillage-1	Gone	**Faecal Spillage (2) 2Gone**

7.3 Appendix 3: Testing procedure for the assessment of microbiological status

Culture: Non-pathogenic *E. coli* strains (from EC1604, EC1605, EC1606, EC1607, EC1608 – strains that contain no known virulence markers for pathogenic *E. coli* are therefore considered to be non-pathogenic)are used. Broth culture of the strains are sent to the abattoir, subcultured and growth in BTSB overnight at 37 °C. On the day of the challenge testing, 5 mL of the culture will be added to 300 mL of sterile BTSB in a sturdy screw-top plastic container to give an inoculum containing around 10 million *E. coli* per mL. The broth culture is stored on ice until its application to the test carcases.

SPC testing procedure: upon sample receival, 1 mL of the sample was diluted in 0.1% peptone salt solution at 10-1 and 10-2 dilution factor, followed by inoculating sample onto the 3M PetrifilmTM Aerobic Count Plate. Plate is then incubated at 35 °C for 48 hrs. All red colonies are counted and reported as cfu/cm2. Reference: AOAC 990.12 Aerobic plate count (Petrifilm)

E.coli / Coliforms testing procedure: upon sample receival, 1 mL of the sample was diluted in 0.1% peptone salt solution at 100 dilution factor for IC studym, and at 10-2, 10-3, 10-4 for CS study, followed by inoculating sample onto the 3M PetrifilmTM EC Plate. Plate is then incubated at 35 °C for 48 hrs. *E.coli* and Coliforms colonies are counted respectively and reported as cfu/cm2. Reference: AOAC 991.14.

Results of the test are obtainable by contacting AMPC on 02 8908 5500 or info@ampc.com.au.