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Application of heat in post cook chillers as a means for *Listeria* reduction in processed meat

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Abstract

A two week baseline survey of GMP sanitation protocols and *Listeria* prevalence in high risk processed meat chillers was performed across three large and four medium to small processed meat facilities in Queensland, Australia. *Listeria* was detected in four of the seven chillers during the initial surveillance testing, with prevalence ranging from 7.8 to 20%. The final *Listeria* prevalence for large chiller A and small chiller B was calculated over a total of six weeks, as these chillers were chosen and available to participate in the heating intervention.

The heating trials consisted of three individual heating interventions followed by two weeks of post intervention sampling to a total of six weeks. The treatment used for small chiller B and large chiller A was 50^{o}C over 2h and 37^{o}C over 36h, respectively. *Listeria* prevalence in small chiller B and large chiller A was reduced to nil (0 /90) from a pre-heating 7.8% (7/90), and to 1.7% (3 /180) from a preheating 10.6% (19/180), respectively. Both reductions were statistically significant at P <0.01.

The incorporation of these two simple chiller heating protocols into facility GMP has effected significant reductions in chiller *Listeria* prevalence. Adoption of these regimes by industry may disrupt the chiller mediated contamination of cooked smallgoods with *Listeria monocytogenes,* and improve the overall safety of processed meat products.

Executive summary

The overall objective of this work was to improve the safety of cooked smallgoods (processed meats) by reducing the contamination with *Listeria monocytogenes*. One of the means by which this contamination process can be disrupted is via reductions in the prevalence of *Listeria* in the environment of post cook chillers.

The four staged objectives of this work were to:

1. Produce a baseline report of the GMP protocols currently used to manage the environmental prevalence of *Listeria* in high risk post cook Queensland chillers
2. Determine the baseline *Listeria* prevalence for these chillers relative to reported GMP protocols.
3. Evaluate a specific *Listeria* minimisation intervention technology in a chiller, in the event that *Listeria* is detected in at least one large meat processor chiller.
4. Evaluate a specific *Listeria* minimisation intervention strategy in a chiller, in the event that *Listeria* is detected in at least one small meat processor chiller.

Details of chillers examined and GMP protocols used to manage the environmental prevalence of *Listeria* are found in Table 1. There were a total of seven producers that took part in the two week post cook chiller GMP and *Listeria* baseline survey. Three of these were large processed meat facilities supplying national retailers, and four were small processors / butchers manufacturing ready to eat meat products. All producers were situated within South East Queensland.

Four of the chillers were blast (intensive) chillers whilst three were holding chillers. Apart from one chiller operating at -3.0^{o}C, the others operated at temperature ranging from 1.5 – 4.5 ^{o}C. Floors were either concrete or epoxy and most contained a drain. The size of the largest chiller was 8.7m x 8.7m x 4.5m and the size of the smallest chiller was 2.4m x 3.2m x 4.5m. Wall panel was composed of insulated expanded polystyrene that was either 75 or 100mm thick.

Apart from the frequency of cleaning, there was little variety in the GMP protocols used to manage the environmental prevalence of *Listeria* in the seven high risk post cook Queensland chillers. The chillers were dry cleaned, and hosed with cold water. A chlorine based foam sanitiser (ranging from 500 - 1000ppm) was applied and manual scrubbing was used. A hot water wash was employed followed by application of a no-rinse quaternary ammonium compound based sanitiser between 200 to 400ppm. The frequency of cleaning varied greatly, ranging from daily to annually; two of the three large facilities sanitised their chillers daily while the other was monthly. Two of the small facilities cleaned weekly, one monthly, and one butcher employed external contractors annually.

Baseline sampling was performed from Monday to Friday on all chillers for 2 weeks. In July 2010, six sites were sampled per large chiller over a five day working week, and three sites were sampled per small chiller over the same time. *Listeria* species were examined using the *Listeria* BAX Automated System and results were reported as Detected or Not Detected /25cm^2. Results are detailed in Table 1.

Listeria was detected in four of the seven chillers, and prevalence ranged from 7.8 to 20%. The two large facilities employing daily cleans (large chillers B & C) did not isolate *Listeria* over the period of analysis. The coldest chiller, operating at -3.0^{o}C likewise did not isolate *Listeria*. The highest joint prevalence was for the annually cleaned small chiller C and small chiller A (20%). Both of these facilities were reluctant to participate in the heating intervention, hence large chiller A and small chiller B were used for further evaluation.

The initial prevalence for large chiller A and small chiller B was calculated over a total of six weeks in order to ascertain statistical significance of intervention changes. The heating trials consisted of three individual heating interventions followed by two weeks of post intervention sampling to a total of six weeks post treatment. Heating was performed primarily on the weekends, either Saturday or Sunday between

4am – 7am. Heaters used were the Hotbox-Axial HBA Fan Blower Heaters HB90415 — 415V 9.0kW and HB15415 415V 15kW (Thermal Electric Elements Pty Ltd, Brisbane, Australia). Heating was effected through the use of the 9kW heating unit (in small chillers) or both units (in large chillers).

Large chiller A was in a bank of 6 blast and 4 holding chillers and hence surrounded by a strong chill load. The heat treatments in that chiller were not able to increase the air temperature to 50°C, but reached and held 37°C for 36h. This time and temperature combination had the effect of completely drying out the chiller. This 'real world' treatment may be an option for processors where a certain time/temperature combination is not possible. *Listeria* prevalence in large chiller A was reduced to 1.7% (3 /180) from an initial 10.6% (19/180) respectively. This reduction was statistically significant and certainly suggests that drying alone can effect a reduction in chiller *Listeria* contamination.

The treatment used for small chiller B was 50°C over 2hrs. *Listeria* prevalence in small chiller B was reduced to nil (0 /90) from an initial 7.8% (7/90). This reduction was statistically significant and mirrors the reductions seen in our earlier work in the frozen meals sector.

Challenges noted (and overcome) in these facilities included the availability of a suitable 3-phase 20 & 32Amp power supply for each of the heating units. The high chill load in concentrated chiller banks was overcome via the application of the lower temperature for a longer time. A further challenge was availability of the chiller for heating (requires consolidation of product into another chiller) and that was difficult to guarantee upon gradually increasing Christmas production pressures.

In summary, this work determined a baseline GMP and *Listeria* persistence survey across seven processed meat chillers, and then evaluated periodic heat treatments as an intervention additional to existing GMP, in order to effect reductions in *Listeria* prevalence. *Listeria* was noted to be present in most chillers in both large and small processors. Statistically significant reductions were effected in both a standard 50°C/2h treatment, as well as a 37°C/36h treatment.

The incorporation into facility GMP of these two simple chiller heating protocols has effected significant reductions in chiller *Listeria* prevalence; adoption of these regimes by industry can potentially disrupt the chiller mediated contamination of cooked smallgoods with *Listeria monocytogenes*, and improve the overall safety of processed meat products.

Table 1. Chiller details and GMP protocols currently used to manage the environmental prevalence of *Listeria*

							Cleaning & Sanitation		*Listeria* detections/n (%)	
Chiller	Processor Type	Chiller Function	Mean Temperature	Dimensions (m) length x width x height	Wall Panel Insulated Expanded Polystyrene (mm)	Floors Material / Drain Present	Frequency	Variation	Pre	Post
Large Chiller A	Large	Blast (Intensive)	4.1	5.0 x 2.8 x 5.0	100	Epoxy / No	Monthly	Nil	19/180 (10.6)	3/180 (1.7)*
Large Chiller B	Large	Holding	2.8	3.6 x 9.0 x 5.0	75	Concrete / Yes	Daily	Nil	0/60 (0.0)	n/a
Large Chiller C	Large	Holding	3.2	4.2 x 7.3 x 5.0	75	Concrete / Yes	Daily	Nil	0/60 (0.0)	n/a
Small Chiller A	Small	Blast (Intensive)	1.5	4.7 x 3.4m 3.9	100	Epoxy / Yes	Weekly	Nil	6/30 (20)	n/a
Small Chiller B	Small	Blast (Intensive)	2.5	8.7 x 8.7 x 4.5	100	Epoxy / Yes	Monthly	Nil	7/90 (7.8)	0/90 (0.0)*
Small Chiller C	Butcher	Holding	4.5	4.5 x 5.0 x 5.0	100	Concrete / Yes	Annually	Contractor	6/30 (20)	n/a
Small Chiller D	Small	Blast (Intensive)	-3.0	2.4 x 3.2 x 4.5	100	Epoxy / Yes	Weekly	Nil	0/30 (0.0)	n/a

Key: * statistically significant reduction

Contents

1. Background

The *Listeria* genus has six species, namely *L. monocytogenes, L. innocua, L. ivanovii, L. seeligeri, L. welshimeri, and L. grayi (10). Listeria monocytogenes* is a non-sporeforming Gram-positive motile foodborne pathogen. Organisms of the *Listeria* genus are widespread throughout nature, being commonly found in the environment and carried by many species of both domestic and wild animals *(19)*. In the environment *Listeria* has been isolated from soils, plant matter, vegetation, silage, river water, surface water, spring water, sewage and both fresh and marine water. *Listeria* has been isolated from 37 mammals, 20 types of birds, frogs, fish, crustacea and ticks *(10)*. 2-6% *(17)* of healthy people shed LM in their feces.

Listeria monocytogenes is the causative agent of epidemic and sporadic listeriosis. *Listeriosis* is clinically defined when the organism is isolated from blood, cerebrospinal fluid, or an otherwise normally sterile site (e.g. placenta, unborn baby). The manifestations of listeriosis include septicaemia, meningitis, encephalitis, and intrauterine or cervical infections in pregnant women, which may result in abortion (2^{nd} / 3^{rd} trimester) or stillbirth. Although some cases occur in individuals without any predisposing condition, most *L. monocytogenes* infections occur in people with suppressed immune systems, ie newborns (<1 year of age), the elderly (>65 years of age), AIDS sufferers and immunocompromised individuals *(10,19).*

Outbreaks of *Listeria monocytogenes* have been associated or linked epidemiologically with the consumption of soft, semi-soft and mould ripened cheese; hot dogs; pork tongue in jelly; processed meats; pate; salami; pasteurized chocolate flavored milk; butter; cooked shrimp; smoked salmon; maize and rice salad; maize and tuna salad; potato salad; raw vegetables; and coleslaw *(10).*

The mortality rate for Listeriosis is between 20-40% for those infected. The significance of *Listeria monocytogenes* to the Australian food industry can not be underestimated. Estimates of annual illnesses due to foodborne Listeriosis in Australia are 26 deaths per year, with 120 hospitalisations and 23 mean days in hospital per patient; the estimated 26 deaths per annum have an estimated present value of A$82.3 million *(2)*. Likewise, Australia's consumer level bacterial recalls have been almost exlusively due to *Listeria monocytogenes* over the past five years *(6).* The annual cost of Australian food safety recalls is estimated at $A14.0 million *(2).* This high mortality rate has led authorities around the world to impose nil *Listeria monocytogenes* standards in ready to eat foods.

Some studies have shown that the risk of disease from foods contaminated occasionally with <100CFU/g is low, even in susceptible populations *(11, 13, 19, 20).* The probability of infection is determined by a number of factors i.e. the number of cells consumed, host specific factors, the type of food and the pathogenicity of the strain. The risk posed by smallgoods is dependent on the initial contamination and the ability of *L.monocytogenes* to grow in these products between the time of manufacture and consumption. In the Australia New Zealand Food Standards Code *(7) t*here is a stated zero tolerance policy for *Listeria monocytogenes* in 5 x 25g samples of packaged cooked cured/salted meats as well as packaged heat treated meat paste and packaged heat treat pâté. Food Standards Australia New Zealand (FSANZ) has additionally published recall guidelines that deal with *Listeria monocytogenes* in ready to eat foods *(5)*. Many processed meats would be expected to undergo a listericidal process (>70^{o}C for 2 min) so these recall guidelines are not necessarily applicable. Nevertheless, these guidelines *contain* action levels of 100 CFU/g for ready to eat foods that exclude the following foods: those that do not require refrigerated storage; those unable to support the growth of *L. monocytogenes;* those not implicated in human listeriosis and those consumed by at risk groups, especially infants. These guidelines specify action levels for recall purposes only and Australian manufacturers must strive for nil tolerance in their packaged ready-to-eat products *(5)*.

Avoiding or minimising cross-contamination is one of the six key hygiene and intervention strategies required to improve the safety of meat (18). The ubiquitous presence of *Listeria monocytogenes*, coupled with its high long-term survival, growth at low temperatures *(10, 20)* and preference for wet surfaces, results in the common occurrence of this pathogen in refrigerators and chilling units *(10)*. The colonization

of post-cook chillers with *L. monocytogenes* may facilitate final product contamination. Recontamination of cooked product is the primary source of *L. monocytogenes* contamination in many commercially produced ready-to-eat (RTE) foods *(20, 21)*; surveys of ready to eat meats repeatedly highlight the isolation of *L monocytogenes* in this category of product.

The main focus when managing *L. monocytogenes* contamination of smallgoods is on preventing contamination by the post-cook factory environment. *Listeria monocytogenes* strains are known to persist within the food processing environment for extended periods of time, 10 years or more in some cases *(15)*. The properties that make a bacterial strain persist are not well understood but are thought to be related to properties such as biofilm formation and elevated resistance to sanitizers *(14, 15)*.
Heat can be used to manage persistent *L. monocytogenes* in the post-cook factory environment, as this pathogen is not unusually heat resistant among vegetative Gram positive bacteria *(12)*. The maximum growth temperature of *L. monocytogenes* is 45^{o}C *(13)* and heat inactivation takes place above that limit, with the rate of inactivation being a function of both time and temperature. Heat has been used, for example, to surface pasteurize and reduce *L. monocytogenes* on vacuum-sealed precooked ready-to-eat meat products *(8)*. Heat can also be applied as steam directly onto surfaces and equipment that need to be sanitized. Of course, the potential of “caking-on” of product needs to be considered individually, based on the particular food matrix. The application of steam onto equipment can be optimized for complex machinery by covering the equipment to be treated with a tarpaulin so as to maximize steam contact time and penetration. Cook-rooms also manage environmental *Listeria* by “pasteurizing” mobile equipment capable of surviving such a heat treatment *(21)*. It has been observed that heating air within a room can be effective for removing moisture at the end of cleaning sanitation *(21)*. Chmielewski and coworkers *(3)* used predictive modeling to suggest that with proper control of time and temperature, hot water sanitation of stainless steel surfaces could serve as an efficient method for elimination of *L. monocytogenes* in biofilms.

An adjunct to the existing Good Manufacturing Practice (GMP) cleaning regimen was therefore sought to reduce smallgood chiller contamination with *L. monocytogenes*. Periodic heat treatment of chillers is an intervention that involves raising the temperature of the chillers to a level which, in combination with the associated drying, may provide multiple stressors and result in a reduction of bacteria present in the chiller. In our previous research *(4)* we tested this hypothesis by applying this heating and drying regimen to post-cook chillers in a frozen meal facility using simple heaters and fans and determining the prevalence of *Listeria* in the chillers before and after implementation of the intervention. Although that study did not completely eliminate *Listeria,* it did dry chillers, was easily taken up into the GMP program, produced no deleterious effects to the treated chillers and significantly reduced environmental post cook chiller *Listeria* contamination.

2. Project objectives

2.1 MLA will receive a baseline report of the GMP protocols currently used to manage the environmental prevalence of *Listeria* in high risk post cook Queensland chillers by a total of seven producers (three large processed meat facilities supplying national retailers and four small butchers manufacturing ready to eat meat products).

2.2 EML will determine a baseline *Listeria* prevalence for these chillers relative to reported GMP protocols. These data will provide an estimate of the scale of the *Listeria* colonisation problem, allow for a basic assessment of the relative efficacy of current GMP protocols with regards to Listeria management, and allow for efficacy assessment in future chiller interventions.

2.3 MLA will receive an evaluation of a specific *Listeria* minimisation intervention technology (thrice fortnightly heat treatment of post cook chillers to an air temperature of 36^{o}C for 37h or until dry) in a high *Listeria* prevalence large meat processor facility.

2.4 MLA will receive an evaluation of a specific *Listeria* minimisation intervention technology (thrice fortnightly heat treatment of post cook chillers to an air temperature of 50^{o}C for 2h or until dry) in a high *Listeria* prevalence small processor / butcher manufacturing ready to eat meats.

2.5 Submission of work to Journal of Food Protection, an International, peer reviewed journal, either as two Research Notes or one Research Article. All IP is owned by MLA and all work is to be approved by MLA pre-submission.

FIGURE 1. Hotbox-Axial Blower Heater HB90415–415V 9.0kW in small chiller B - wet floor pre heating.

3. Methodology

Selection of meat processing facilities

Large Queensland smallgoods processing facilities known to the authors to be suppliers to large national retailers were approached and requested to take part in the current work. Those that accepted were further qualified by an onsite assessment confirming that the offered post cook chillers were infact adequately separated from raw product and exposed product did have the potential to be contaminated by chiller resident *Listeria* species (and hence would benefit from a *Listeria* reduction intervention). These chillers were arbitrarily designated as large chillers A, B & C.

Similarly, butchers manufacturing ready to eat meats were approached, as were small to medium smallgoods manufacturers; it is worth noting that onsite qualification disqualified six candidate manufacturers due to inadequate chill segregation of cooked from raw product – in most cases raw product was present within a few feet of cooked product and although there were attempts made to segregate raw and cooked within the same chiller, an intervention aimed at reducing persistent *Listeria* colonisation is not indicated in a situation when a contamination source is routinely present. Those chillers that were qualified were arbitrarily designated as small chillers A, B, C & D.

Assembly of multi-discipline team.

Upon qualification, the researchers assembled a multi-discipline team at facility level with whom the project objectives and anticipated outcomes were explained. Site inductions, chiller access, accompanying staff and reporting timeframes were organized via this team. In the larger sites, engineering, operations and quality assurance teams were concurrently engaged in the baseline chiller surface monitoring program, as well as the heat treatment planning (pending results). The involvement of staff additional to quality assurance ensured that project support was maintained at a high level.

In smaller sites, the team engaged was smaller and inductions, chiller access and accompanying staff were not always required. It is interesting that although the project was explained, including the requirement to treat chillers should they present with the highest *Listeria* isolation rate, the downstream refusal of two of the small facilities to undergo this heat treatment may indicate an underlying discomfort and confidentiality concern regarding the presence of the pathogen on site.

GMP survey.

A baseline survey of the GMP protocols used to manage the environmental prevalence of *Listeria* in high risk post cook chillers was verbally administered by the authors of this report. The questions asked were:

1. Is the chiller used primarily as a blast (intensive) or hold chiller?
2. What is the mean set temperature of this chiller?
3. What are the dimensions of this chiller?
4. What is the thickness of the insulated expanded polystyrene wall panel?
5. What is the chiller Floor material?
6. Is there a chiller present in the chiller?
7. How frequently do you clean and sanitise this chiller?
8. Brief summary of cleaning and sanitation procedure please.

Sampling and microbiological analysis

Initial baseline sampling was performed on all chillers in July 2010. The entire project stretched to October 2010. All chillers were used during sampling and ran completely through production. Chillers were

sampled for *Listeria* species from Monday to Friday over a two week period. Six sites were sampled from each large chiller, and three sites from each small chiller. Over two weeks each of the three large chillers had 60 swabs taken, and each of the small chillers had 30 swabs taken. The areas targeted were internal areas floors, walls, drains, frames, bollards, seals and doors. Separate Transwab® Amies Transport Swabs Swabs (Transwab® with modified Amies without charcoal, MWE Medical Wire & Equipment, Wiltshire, England) were used to sample an area of approximately 25 cm2.

Swabs were tested for the presence of *Listeria* using the *Listeria* BAX Automated System (DuPont Qualicon, Wilmington, DE, USA). Each swab was enriched in 9 ml of buffered *Listeria* enrichment broth (Amyl Media, Melbourne, Australia) for 24 h at 35°C. 1 ml of enrichment was inoculated into 10mL MOPS-buffered *Listeria* enrichment broth (Amyl Media) and incubated at 35°C for 18 – 24 h. Enrichment cultures were analyzed using the automated PCR, following the manufacturer's user's guide for preparing re-agents, performing the test, and reading the results. Specifically, enrichment cultures were lysed and the lysate was used to hydrate the PCR reagents contained within a proprietary tablet. Processing in the automated PCR unit took approximately 4 hours, and electronic results appear as positive/negative icons on the unit screen. Presumptive positive samples were confirmed following manufacturer's instructions by streaking retained MOPS-buffered *Listeria* enrichment broth onto Oxford and PALCAM agar (Amyl Media) and incubating at 37oC for 48 h. Colonies surrounded by dark brown or black haloes were confirmed as per the Australian Standard method AS1766.2.16 *(1)*. Results were reported as *Listeria* spp. detected or not detected/25 cm2.

The sampling was completed for *Listeria* species only. It would have been possible to look specifically for *L. monocytogenes*, but the additional resources required to perform this testing was not in line with its key outcomes, namely to evaluate the hypothesis that heating smallgoods chillers reduces the presence of all *Listeria* species. In previous work at the state of Queensland we ascertained from 456 *Listeria* species isolates collected from routine food quality and environmental monitoring schemes that the component of *Listeria monocytogenes* within these isolates was 50.4% (*9*).

The initial prevalence for large chiller A and small chiller B was calculated over a total of six weeks in order to help ascertain statistical significance of intervention changes. In total, 180 pre heating swabs were drawn from large chiller A, and 90 pre heating swabs from small chiller B.

The heating trials consisted of three individual air heating interventions followed by two weeks of post intervention sampling to a total of six weeks post treatment. Heating was performed primarily on the weekends, commencing Saturday or Sunday between 4am – 7am. Heaters used were the Hotbox-Axial HBA Fan Blower Heaters HB90415 — 415V 9.0kW and HB15415 415V 15kW (Thermal Electric Elements Pty Ltd, Brisbane, Australia). Heaters were modified by mounting them onto mobile stands, fitting them with a 10 m × 3 phase cable and installation of a 20A plug top with thermostat/auto cut-off designed to switch the unit off at the target temperature. Heating was effected through the use of the 9kW heating unit (in small chillers) or both units (in large chillers).

Product was consolidated into a different holding chiller allowing for implementation of the heating regimen at the end of production and sanitation. Empty crates, mobile racking and pallets were allowed to remain in the chillers. Circulating refrigerant valves within chilling units were released prior to operation of heaters to minimize heat-induced refrigerant pressure build up.

Large chiller A was in a bank of 6 blast and 4 holding chillers and hence surrounded by a strong chill load. The heat treatments in that chiller were not able to increase the air temperature to 50°C, but reached and held 37°C for 36h. The treatment used for small chiller B was 50°C over 2hrs.

In total, 180 post heating swabs were drawn from large chiller A, and 90 heating swabs from small chiller B. The same sites sampled in the initial baseline were sampled post heating in order to compare pre and post *Listeria* prevalence. A grand total of 750 environmental samples were drawn over this survey.

Statistical analysis

The relationship between *Listeria* prevalence and chiller intervention was analyzed using the CHITEST formula in Microsoft Excel 2003. Significance was indicated when $P < 0.01$.

FIGURE 2. Hotbox-Axial Blower Heater HB90415–415V 9.0kW in small chiller B - dry floor post heating.

4. Results and discussion

4.1 GMP survey & baseline *Listeria* prevalence

All facilities provided survey answers and results are tabulated in Table 1. Four of the participating chillers were blast (intensive) chillers whilst three were holding chillers. Apart from one chiller operating at -3.0^{o}C, the others operated at temperatures ranging from 1.5 – 4.5 ^{o}C. Floors were either concrete or epoxy and most contained a drain. The size of the largest chiller was 8.7m x 8.7m x 4.5m and the size of the smallest chiller was 2.4m x 3.2m x 4.5m. Wall panel was composed of insulated expanded polystyrene that was either 75 or 100mm thick.

A common theme was apparent when manufacturers were asked to briefly summarise their cleaning and sanitation procedures. Apart from the frequency of cleaning, there was little diversity in the GMP protocols used to manage the environmental prevalence of *Listeria* in these seven high risk post cook Queensland chillers. All chillers were dry cleaned, and hosed with cold water. A chlorine based foam sanitiser (ranging from 500 - 1000ppm) was applied and manual scrubbing was used. A hot water wash was employed followed by application of a no-rinse quaternary ammonium compound based sanitiser between 200 to 400ppm. The frequency of cleaning did vary greatly, ranging from daily to annually; two of the three large facilities sanitised their chillers daily while the other was monthly. Two of the small facilities cleaned weekly, one monthly, and one butcher employed external contractors annually (no data available on those cleaning and sanitation procedures).

Listeria was detected in four of the seven chillers, and prevalence ranged from 7.8 to 20%. A breakdown of the *Listeria* prevalence per sampling site in non heated chillers is contained in Table 2. The sites most commonly yielding *Listeria* are drains and door seals followed by floors.

It is noteworthy that the two large facilities employing daily cleans (large chillers B & C) did not isolate *Listeria* over the period of the analysis. The reason sited by quality personnel for applying a daily clean was heavy use of those particular chillers during production; it must be noted that similar heavy use was noted in other chillers that were utilising only a weekly, monthly or annual clean. The nature of *Listeria* chiller contamination is such that this ubiquitous organism is constantly re-introduced and management relies upon vigilance in preventing this re-introduction establishing into a niche *(21)*; disruption of this establishment mechanism may be the mechanism of control in the daily sanitised chillers.

The coldest chiller, small chiller D, operating at -3.0^{o}C likewise did not yield *Listeria* over the period of the analysis. It would be expected that a super-chilled room would not harbour *Listeria*. Indeed *L. monocytogenes* does not grow below -0.4 ^{o}C *(5)* and holding of any organism outside of its growth parameters effects a slow inactivation. The reason for qualifying small chiller D (an additional chiller to the three initially scheduled for monitoring) was the observation during onsite qualification that this small blast chiller was subject to a heavy chilling load (chiller temperature rose to 10 ^{o}C for extended periods of time) and that the overall chiller environment was extremely humid with floor puddles accumulating. Regardless of these high temperatures and moist environment, the super chilling temperature effectively managed any ongoing *Listeria* re-introduction.

The highest prevalence noted in the baseline survey was for the annually cleaned small chiller C and small chiller A (both 20%). As demonstrated in the case of a high frequency (daily) clean very adequately managing incoming contamination, a low frequency (annual) clean did not. Small chiller A, which also presented with a 20% prevalence was cleaning on a more frequent (weekly) basis; however the high prevalence may be related to the observation that on two of the ten occasions that the authors were drawing samples *in the chillers,* a staff member, colour coded with clothing restricted to the raw area, ingressed into the high risk chiller. Personnel traffic issues are a challenging yet essential component of GMP which must be in place as a basic prerequisite in managing *Listeria* in smallgoods chillers.

Table 2. *Listeria* prevalence per sampling site in non-heated chillers.

Sampling Site	*Listeria* detections / *n* (%) per chiller				
	Large B	Large C	Small A	Small C	Small D
Drain	0/8 (0.0)	-	2/10 (20)	2/5 (40)	0/8 (0.0)
Door Frame	0/8 (0.0)	0/9 (0.0)	-	-	
Door Seals	0/7 (0.0)	0/10 (0.0)	3/10 (30)	1/5 (20)	0/8 (0.0)
Door & Hinges	0/6 (0.0)	0/6 (0.0)	-	-	
Floor	0/10 (0.0)	0/11 (0.0)	0/5 (0.0)	3/8 (37.5)	0/5 (0.0)
Floor Pallet	-		1/5 (20)	-	-
Floor/Wall Coving	0/9(0.0)	0/9 (0.0)	-	-	-
Wall/Chilling Unit	0/12 (0.0)	0/17 (0.0)	-	0/3 (0.0)	0/2 (0.0)
Overhead Framework	-	-	-	0/9 (0.0)	0/2 (0.0)
Bollard	-	-	-	-	-
Hanging Curtains	-	-	-	-	0/5 (0.0)
TOTAL	0/60 (0.0)	0/60 (0.0)	6/30 (20)	6/30 (20)	0/30 (0.0)

4.2 Chiller Heating

Listeria prevalence per chiller sampling site in heated chillers pre- and post-chiller intervention is presented in Table 3. As per the other chillers, the most persistently contaminated areas are door seals, drains and floors. Overhead framework, bollards, hanging curtains and walls, did not commonly carry *Listeria* (this may be related to the relatively high moisture of a drain or door seal when compared to dry overhead framework and bollards). Our intervention aims to reduce the high moisture of drains and seals.

Large chiller A was in a bank of 6 blast and 4 holding chillers and hence surrounded by a strong chill load. The heat treatments in that chiller were not able to increase the air temperature to 50^{o}C, but reached and held 37^{o}C for 36h. This time and temperature combination had the effect of completely drying out the chiller. This 'real world' treatment may be an option for processors where a certain time/temperature combination is not possible. *Listeria* prevalence in large chiller A was reduced to 1.7% (3 /180) from an initial 10.6% (19/180) respectively. This reduction was statistically significant and certainly suggests that drying alone can effect a reduction in chiller *Listeria* prevalence. Of course due to the nature of the constant re-introduction of *Listeria* in the processing environment (as noted in the earlier case of uncontrolled personnel traffic), it is not necessarily feasible that *Listeria* can be completely eliminated.

The treatment used for small chiller B was 50^{o}C over 2hrs. *Listeria* prevalence in small chiller B was reduced to nil (0 /90) from an initial 7.8% (7/90). This reduction was statistically significant and mirrors the reductions seen in our earlier work in the frozen meals sector. It is testament to the management of small chiller B that upon seeing the post heating reduction effected in the chiller, heating is being introducing not only in their post cook chillers but also on their high risk processing floor. It is directly due to the validation of a gentle temperature over an extended time which has allowed processing areas that contain temperature sensitive processing equipment to consider a *Listeria* heating intervention that may otherwise have been limited to chillers and other areas not containing heat sensitive equipment.

Table 3. *Listeria* prevalence per sampling site in heated chillers

Sampling Site	*Listeria* detections / *n* (%) Large Chiller A		*Listeria* detections / *n* (%) Small Chiller B	
	Pre	Post	Pre	Post
Exposed Drill Hole	-	-	0/3 (0.0)	0/3 (0.0)
Drain	-	-	3/21 (14.3)	0/21 (0.0)
Door Frame	3/26 (11.5)	1/26 (3.8)	-	-
Door Seals	9/22 (40.9)	1/22 (4.5)	0/7 (0.0)	0/7 (0.0)
Door & Hinges	2/23 (8.7)	1/23 (4.3)	0/3 (0.0)	0/3 (0.0)
Floor	2/27 (7.4)	0/27 (0.0)	2/21 (9.5)	0/21 (0.0)
Floor/Wall Coving	2/27 (7.4)	0/27 (0.0)	2/19 (10.5)	0/19 (0.0)
Wall	1/28 (3.6)	0/28 (0.0)	0/5 (0.0)	0/5 (0.0)
Overhead Framework	0/20 (0.0)	0/20 (0.0)	0/11 (0.0)	0/11 (0.0)
Bollard	0/7 (0.0)	0/7 (0.0)	-	-
TOTAL	19/180 (10.6)	3/180 (1.7)	7/90 (7.8)	0/90 (0.0)

4.3 Challenges of implementation

Challenges noted (and overcome) in implementing a two-weekly GMP chiller heating regime included the availability of a suitable 3-phase 20 & 32Amp power supply for each of the heating units. Although these power supplies are commercial standards and found in abundance over the general processing areas, they are less frequently found in chillers. Engineering, consulted pre commencement, arranged wiring.

Inability to raise chiller air temperature in concentrated chiller banks (large chiller A), was overcome via the application of the lower temperature for a longer time principle. The fact that a significant reduction was noted on a dried chiller in the absence of any temperature stress, supports the premise that dessication pressure alone may be sufficient for a reduction in *Listeria* colonization rates. This 'real world' treatment may be an option for processors where a certain time/temperature combination is not possible.

A further and perhaps more dire challenge was availability of the chiller for heating (heating requires an empty chiller and hence consolidation of product into another chiller). Space in another chiller was difficult to guarantee upon gradually increasing Christmas production pressures. As these pressures were being felt as early as October, it is certainly the case that unless planning for significant chiller redundancy does not take place, chiller heating arrangements may well be suspended upon the industry's traditionally heavy peak production months of November and December. Ironically, it will be at these times that this treatment would effect the greatest benefit.

5. Success in achieving objectives

5.1 MLA will receive a baseline report of the GMP protocols currently used to manage the environmental prevalence of *Listeria* in high risk post cook Queensland chillers by a total of seven producers (three large processed meat facilities supplying national retailers and four small butchers manufacturing ready to eat meat products).

This baseline report has been completed and discussed in section 4.1 under Table 1. Indeed this objective has been exceeded as the objective stated a total of six chillers, and we added a seventh for GMP assessment within the same resource base.

5.2 EML will determine a baseline *Listeria* prevalence for these chillers relative to reported GMP protocols. These data will provide an estimate of the scale of the *Listeria* colonisation problem, allow for a basic assessment of the relative efficacy of current GMP protocols with regards to Listeria management, and allow for efficacy assessment in future chiller interventions.

This baseline report has been completed and was also discussed in section 4.1 under Table 1 and Table 2. Indeed this objective has been exceeded as the objective stated a total of six chillers, and we added a seventh for Listeria testing within the same resource base.

5.3 MLA will receive an evaluation of a specific *Listeria* minimisation intervention technology (thrice fortnightly heat treatment of post cook chillers to an air temperature of 36^{o}C for 37h or until dry) in a high *Listeria* prevalence large meat processor facility.

This evaluation has taken place and is fully discussed in section 4.2 and Table 3. Indeed the temperature of chiller evaluation has been of necessity reduced to 36^{o}C for 37h, a shorter temperature over a longer time. This protocol has nonetheless succeeded in reducing Listeria and provides industry with an additional option. This gentle treatment has the advantage of (a)application in a strong chill area, (b) application to areas containing heat sensitive equipment and (b) minimizing heating preparation work as chilling gas does not need to be evacuated pre heating.

5.4 MLA will receive an evaluation of a specific *Listeria* minimisation intervention technology (thrice fortnightly heat treatment of post cook chillers to an air temperature of 50oC for 2h or until dry) in a high Listeria prevalence small processor / butcher manufacturing ready to eat meats.

This evaluation has taken place and is also fully discussed in section 4.2 and Table 3.

5.5 Submission of work to Journal of Food Protection, an International, peer reviewed journal, either as two Research Notes or one Research Article. All IP is owned by MLA and all work is to be approved by MLA pre-submission.

This report will form the basis for the submission to Journal of Food Protection. We are on target to submit before December 1, 2010. Indeed the development of an additional time / temperature combination has improved the appeal of the yet to be submitted paper.

6. Impact on meat and livestock industry – Now and in five years time

Minimising cross-contamination is one of the six key hygiene and intervention strategies required to improve the safety of meat *(18)*. Reducing the post cook chiller prevalence of *Listeria* reduces contamination of ready to eat meats and results in a safer product.

By utilizing **NOW** a very simple GMP based intervention, currently in use with frozen meals, the Australian red meat industry will be managing food safety of ready to eat meats which is the subject of customer concern (and purchasing activity) and negative health department advice; high risk populations are advised to avoid consumption of cold meats whilst the results of ongoing *Listeria monocytogenes* surveys by state regulators is used to defend such advice. The immediate success of the intervention to participating processors can be measured easily in terms of pre and post *Listeria* prevalence in high prevalence post cook chillers.

Longer term, **IN FIVE YEARS,** if a successful intervention was to be implemented across the industry, reduction of chiller mediated post cook contamination would result in a reduction of *Listeria* detections across industry and state regulator surveys, reduction of *Listeria monocytogenes* recalls, as well as potentially increased customer acceptance and changes in purchasing behaviour. As the acceptability and reputation of processed meats further improves, there will be a direct benefit to all stakeholders in the industry.

7. Conclusions and recommendations

7.1 PRIMARY Conclusions and Recommendations - Application of heat in post cook chillers as a means for *Listeria* reduction in processed meat

7.1.1 We suggest that periodic heat treatment of 50°C/2h as an intervention additional to existing GMP, will effect a statistically significant reduction in smallgood chiller *Listeria* prevalence. Adoption of this regime by industry can potentially disrupt the chiller mediated contamination of cooked smallgoods with *Listeria monocytogenes*, and improve the overall safety of processed meat products.

7.1.2 We suggest that periodic heat treatment of 37°C/36h as an intervention additional to existing GMP, will effect a statistically significant reduction in smallgood chiller *Listeria* prevalence. Adoption of this regime by industry can potentially disrupt the chiller mediated contamination of cooked smallgoods with *Listeria monocytogenes*, and improve the overall safety of processed meat products.

7.2 SECONDARY Conclusions and Recommendations - GMP Baseline Survey

7.2.1 We observe that annual cleaning and sanitation of post cook chillers may not be a sufficiently intensive frequency in preventing elevated *Listeria* prevalence in post cook chillers.

7.2.2 We observe that daily cleaning and sanitation of post cook chillers may be a highly effective frequency in preventing elevated *Listeria* prevalence in post cook chillers.

7.2.3 We observe that maintaining super chilled post cook chillers (-3°C) may be an effective strategy in preventing elevated *Listeria* prevalence in post cook chillers.

7.2.4 We observe that GMP lapses such as personnel traffic from raw to cooked areas undermine the prerequisite programmes necessary to prevent elevated *Listeria*
prevalence in post cook chillers.

We have described two protocols to potentially reduce *Listeria* contamination in the post processing chiller environment. Certainly a limitation of this technique is the required redundancy of chillers, and it is recognized that many facilities do not operate with such a redundancy. Although this protocol is unable to completely eliminate *Listeria,* it does dry chillers, is easily taken up into the GMP program, produces no deleterious effects to the treated chillers and has significantly reduced environmental post cook chiller *Listeria* contamination.

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9. Appendices

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9.2 Chiller Photographs

FIGURE 3(a) Portable Racks and **(b)** Floor pallets & crates in small chiller B – Pre Heat Treatment.

FIGURE 4. (a) Post cook holding corridor leading to large chiller A on left **(b)** blast chiller A (fully open).

FIGURE 4: (a) Large chiller A showing bollards, floor wall coving with exit door open to holding chiller with product in the distance and **(b)** same chiller with exit door shut and showing overhead framework.

FIGURE 5: (a) 32 Amp heater connection (hard to find switch near chillers!) **(b)** hanging curtains for small chiller D.