agresearch Farming, Food and Health. First

Te Ahuwhenua, Te Kai me te Whai Ora. Tuatahi

Effect of peroxyacetic acid-based carcass wash on the onset of 'blown pack' spoilage of vacuum-packed chilled venison

July 2006

CR 1121

2020 SCIENCE

New Zealand's science. New Zealand's future.

agresearch

Client Report - CR 1121

Effect of peroxyacetic acid-based carcass wash on the onset of 'blown pack' spoilage of vacuum-packed chilled venison

Prepared for DEEResearch

S.L. Urlich, K.H. Adams, C.P. Bueno, J.A. Boerema, D.M. Broda & G. Brightwell

July 2006

Inquiries or requests to:
Shelley Urlich
Email: Shelley.urlich@agresearch.co.nz
Food, Metabolism & Microbiology, AgResearch Ltd
Private Bag 3123, Hamilton, New Zealand

Mike North
Food, Metabolism & Microbiology
Food & Health

Summary

This study investigated individual process variables in the context of earlier research indicating that peroxyacetic acid-based carcass wash (Inspexx^{200}) is capable of *in vitro* inactivation of *Clostridium estertheticum* spores. The variables investigated were:

a) two levels of *Cl. estertheticum* spores (0 and 260 spores per cm^2);
b) three post-packaging storage temperatures (-1.5, 0 and 2°C), and
c) two pre-packaging meat rinses (water and Inspexx^{200}).

The results of this multivariant 60-pack experiment were statistically analysed using Genstat version 5.42. The major findings were that the presence of 260 spores per cm^2 decreased by two thirds the nominal shelf life of vacuum packed chilled venison. Treatment with Inspexx^{200} delayed the onset of pack blowing in packs carrying high inoculum stored at -1.5°C, but not in packs stored at 0 or 2°C.

The results of the present study indicate that the Inspexx^{200} treatment trialled will not eliminate the spoilage threat posed by clostridial 'blown pack' spoilage spores present on meat surfaces. In practical terms, the present study points strongly to the adoption of best practices to delay or prevent the onset of 'blown pack' spoilage within a reasonable commercial chilled storage period. The recommended best practice would use stringent process hygiene at dressing and pelt removal to minimise the initial contamination of carcass meat with spores of 'blown pack'-causing clostridia. Following packaging, the temperature of the product should be reduced to -1.5°C and held at that temperature to as close to the point of retail sale/end use as is practicable. When combined with final product storage temperature of -1.5°C, Inspexx^{200} rinse can be recommended for an extension of shelf-life of venison cuts contaminated with *Cl. estertheticum* spores.

Contents

1. Introduction

'Blown pack' spoilage is characterised by copious gas production in vacuum packs, leading to gross pack distension during storage at temperatures between -1.5 and 2°C. This spoilage condition is caused by psychrophilic clostridia which appear to enter meat processing plants on pelts and/or in faeces of slaughter animals (Broda *et al*. 2002, Boerema *et al*. 2003). It is believed that means for controlling 'blown pack' spoilage in a meat plant can be afforded with the development of technologies to remove, kill or inactivate spores of psychrophilic clostridia present on dressed carcasses.

To date, little information exists on the efficacy of carcass decontamination technologies and/or agents against spores of 'blown pack'-causing clostridia. A previous study investigated the effect of peroxyacetic acid-based carcass wash Inspexx^{200}, heat and ultrasonic waves on the survival of *Clostridium estertheticum* spores *in vitro*. While the survival of *Cl. estertheticum* spores following heat treatment was significantly affected by temperature, complete inactivation of approx. 4 to 5 log cfu ml^{-1} spores required a severe treatment consisting of an exposure to 100°C for 240 s (Broda 2006). In contrast, *in vitro* inactivation of the same spore load was readily obtained in spore preparations that underwent treatments with peroxyacetic acid-based agent, Inspexx^{200}.

The aim of this study was to determine whether complete or partial inactivation of *Cl. estertheticum* spores with peroxyacetic acid-based carcass wash can be attained in the venison meat model.

2. Objectives

- To determine whether peroxyacetic acid-based carcass rinse can delay the onset of gas production in chilled vacuum-packed venison inoculated with spores of *Cl. estertheticum*.

- To establish whether the inoculum level and storage temperature influence the time-to-onset and the severity of 'blown pack' spoilage of vacuum-packed chilled venison cuts.

3. Materials and Methods

3.1 Microorganisms

The reference strain of *Cl. estertheticum* DSM 8809^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. This strain was revived in sterile anaerobic milk and was subcultured onto Columbia Blood Agar (CBA, Oxoid) containing 5% v/v sheep's blood. The strain was grown anaerobically at 10°C for 21 days.

Before being used in spore preparation, the strain was checked for purity by plating onto CBA agar.

3.2 Spore Preparation

Spores were produced in pre-reduced Peptone, Yeast Extract, Glucose, Starch (PYGS) broth (Lund *et al.* 1990). A volume (500 ml) of PYGS broth in a culture bottle was inoculated with an exponentially growing culture of *Cl. estertheticum* DSM 8809^T and incubated anaerobically at 10°C for a minimum of 6 months to promote sporulation.

A spore suspension was prepared when a typical spore appearance, presence of refractive spheres, was observed with phase contrast microscopy in the sporulating culture. The culture was centrifuged, washed twice with ice-cold saline (0.85% NaCl) and resuspended in 5 ml of ice-cold saline. The spore suspension was heated at 60°C for 10 min to eliminate any remaining vegetative cells. Spore suspensions were stored frozen at -18°C until required for inoculum preparation. Since conditions in a saline suspension do not induce activated spores to germinate, the spores revert to a dormant state during frozen storage.

Serial dilutions of spore suspension were plated onto CBA agar and incubated anaerobically at 7°C for 5 weeks to determine an approximate number of colony forming units (cfu). For inoculation, spore suspensions were prepared in sterile saline to give approx. 10^5 cfu ml^{-1}.

3.3 Meat Substrate

Hot boned venison flaps were obtained immediately after boning and transferred to the laboratory. The meat was cut into 20 x 15 cm pieces (approx. surface area 300 cm^2), and individual pieces were pinned onto polystyrene board covered with sterile polyethylene. Five replicate samples were prepared for each treatment type (water and Inspexx^{200}), inoculum level (no inoculum and high inoculum) and storage temperature (-1.5, 0, and 2°C).

3.4 Inoculation

For the high inoculum, replicate samples were each inoculated with a volume (0.2 ml) of 10^5 cfu ml^{-1} spore suspension to obtain a final load of 260 spores per cm^2. Those labelled as no inoculum each had a volume (0.2 ml) of sterile water added. With all replicates, the spore or water inoculum was spread evenly over the meat surface and left to absorb at room temperature for aprox.10-15 minutes.

3.5 Treatment

Inoculated meat samples were secured vertically onto a polystyrene wall of a spray cabinet (Figure 1), awaiting spray application.

Figure 1.
A photograph of a spray applicator used in the study to deliver water or Inspexx^{200} rinse to inoculated meat samples.

The spray applicator consisting of 3 spray nozzles located 28 cm apart on the rotating delivery tube was used to give full spray coverage of samples. A widen pump with a pulse dampener was set to deliver an even pressure of 6 bar at the spray nozzle. A single row of 3 samples placed at the level of the central nozzle was sprayed at a time. At the time of application the distance between the central spray nozzle and each meat sample was 30 cm.

Replicates allocated for water treatment were sprayed first using potable water according to the procedure just described. For peroxyacetic acid-based rinse, Inspexx^{200} (Ecolab) was diluted in potable water to achieve final concentration of 180 ppm according to the manufacturer's instructions. The concentration of the ready-to-use product was checked with a titration kit supplied by the manufacturer. Inspexx^{200} was applied onto meat samples in the exact same manner as the water. Both treated and untreated meat samples were left to dry at room temperature for 30 min. Following this drying period samples were packaged immediately.

3.6 Packaging and Storage

Samples were placed individually into pre-labelled vacuum bags (BB7L; Cryovac). For all vacuum packs, bag sizing consistent with the cut size was employed. Vacuum packs (60 in all) were immediately packed using a Securepak 10 Controlled Atmosphere Packaging Machine (Securefresh Pacific). Sealed packs were subjected to a heat shrinking treatment consisting of the total immersion of packs in 82°C water for 7 to 8 seconds.

The sealed packs were placed into chilled storage at -1.5, 0 and 2°C. Operating temperatures of the storage chillers were monitored using KOOLTRACK data loggers (Kooltrack Inc., USA).

3.7 Monitoring of the Vacuum-Packs

Packs were examined regularly for the presence of gas bubbles in the drip and their current pack distension status was recorded as a score from 0 to 5. Score 0 was assigned to packs with no gas bubbles in the drip; score 1 was assigned to packs that had small gas bubbles in the drip; score 2 was assigned to packs at the 'loss of vacuum' stage; score 3 was assigned to obviously 'blown', puffy packs; score 4 was assigned to fully distended packs without tightly stretched packaging; and score 5 was given to tightly stretched, 'overblown' packs. It should be noted that in commercial practice packs with a distension score of 1 would not be recognised as being abnormal.

The monitoring was terminated at 106 days. On conclusion of the trial, representative packs of each treatment/inoculum/storage temperature combination were massaged thoroughly, opened aseptically and a volume (approx. 1 ml) of the exudate was collected for DNA extraction and PCR analysis.

3.8 Statistical Analyses

Time to the onset of 'blown pack' spoilage (the variate being the day when score is no longer 1), pack distension status on arrival at overseas marketplace (the

variate being the score at 43rd day of storage) and pack distension status near the end of expected shelf-life (the variate being the score at 89th day of storage) were analysed using Genstat version 5.42 (Lawes Agricultural Trust). With all three indicators, means of five replicate packs for each temperature and treatment were used. With analysis of time to the onset of 'blown pack' spoilage, packs that did not show any gas production by the time the trial concluded were assigned value 106. Because of the skewed nature of the values of the day that each pack first exceeded a score of 1, this data was log transformed before analysis; also the packs that never exceeded 1 during the course of the measurements were treated as censored values in the analysis. Too many censored values were encountered in the analysis, consequently, analysis of variance (ANOVA) was used to establish the effect of storage temperature and treatment on time to onset of pack blowing with packs carrying high inoculum only. With uninoculated packs the Fischer Exact test was used to establish the effect of storage temperature and treatment on percentage of packs blown at day 53, by which time all main effects of the trial variables were clearly visible. Similarly, to establish effect of inoculum Fischer Exact test was applied to percentage of packs blown at day 53.

With both uninoculated and inoculated packs ANOVA was successfully used to analyse the effect of storage temperature, treatment and inoculum on day 43 and 89 scores.

3.9 Molecular Detection of *Cl. estertheticum*

The detection of *Cl. estertheticum* in the exudates was conducted according to methods described previously (Broda *et al.* 2003). Primers EISRF and EISRR targeting the 16S-23S rDNA internal transcribed spacer fragment of *Cl. estertheticum* were used. Amplified PCR products were visualised with ethidium bromide by UV transillumination.

4. Results

Except during defrost cycles, temperature in the -1.5°C chiller was maintained within 1.0°C of the set point, while temperatures in the 0 and 2°C chillers were maintained between -1.0 and 1.0°C, and between 1.5 and 3.0°C, respectively. Following defrost cycles, set point temperatures were re-established within 2 h of the start of each cycle. Due to late battery failure no data was logged for the final 14 days of the 106 day trial with 2°C packs. However, no chiller failure was experienced during this time and subsequent temperature monitoring with this chiller confirmed that it was operating within the set parameters.

Means of replicate results for times to the onset of 'blown pack' spoilage of vacuum packed venison stored at -1.5, 0 and 2°C are shown in Table 1. Similarly, means of replicate pack distension scores at 43- and 89-days storage are shown in Table 2 and Table 3, respectively. Storage profiles showing the effect of trial variables on pack distension status and the onset of gas production in vacuum-packed chilled venison are presented in Figure 2.

Table 1. Mean times (in days) to the onset of blowing of vacuum packed venison that underwent pre-packaging wash with either water or Inspexx^{200}, during its subsequent storage at -1.5, 0 and 2°C. The onset of blowing was defined as time when the score for pack distension was no longer 1. Values represent means of five replicates.

Inoculum level	Storage temperature	Type of wash	
		Water	Inspexx^{200}
None	-1.5	99	95
None	0	≥106	≥106
None	2	83	39
High	-1.5	39	66
High	0	47	46
High	2	28	30

Table 2. Mean scores representing pack distension status of either water or Inspexx^{200} – treated vacuum-packed venison at 43 days storage at -1.5, 0 and 2°C. Values represent means of five replicates.

Inoculum level	Storage temperature	Type of wash	
		Water	Inspexx^{200}
None	-1.5	0	0
None	0	0	0
None	2	0.8	2.6
High	-1.5	2	1.6
High	0	2.4	1.2
High	2	2.2	3.2

Table 3. Mean scores representing pack distension status of either water or Inspexx^{200} – treated vacuum-packed venison at 89 days storage at -1.5, 0 and 2°C. Values represent means of five replicates.

Inoculum level	Storage temperature	Type of wash	
		Water	Inspexx^{200}
None	-1.5	1.2	1
None	0	0	0.2
None	2	1.6	3
High	-1.5	3.4	2.4
High	0	3	4.4
High	2	3.6	4

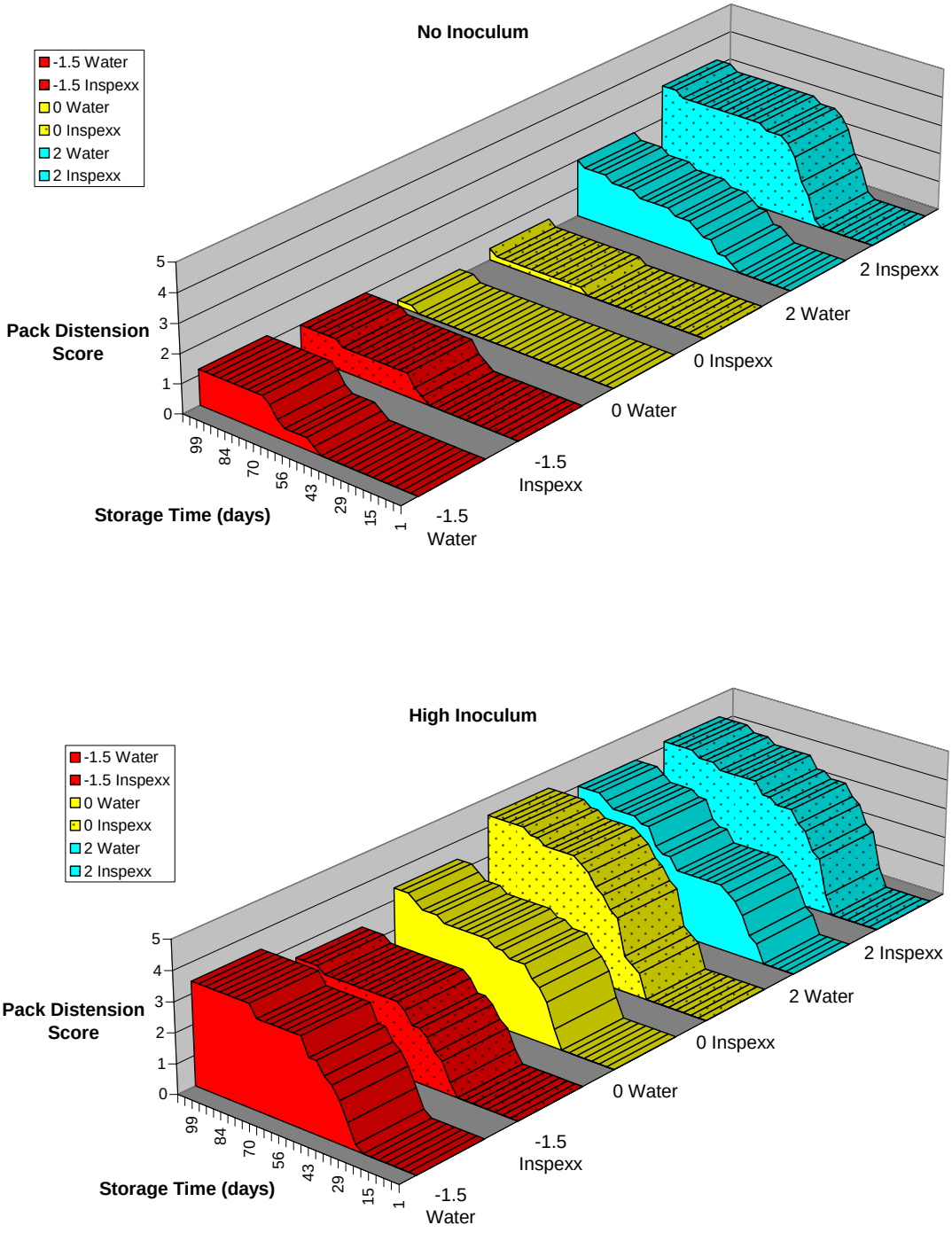

Figure 2. The influence of inoculum level, storage temperature and treatment type on pack distension status and the onset of 'blown pack' spoilage in vacuum-packed venison cuts. Meat samples were treated with either water (solid bars) or Inspexx^{200} (patterned bars) and were stored at -1.5, 0 and 2°C. The data are means of five replicates.

4.1 Influence of Inoculum Level

Statistical analysis by Fischer Exact test showed that inoculum level had significant effect ($P < 0.001$) on percentage of packs blown by day 53. The longest mean times to the onset of blowing were obtained for uninoculated packs and shelf life was as short as 26 days in vacuum packs carrying high inoculum. Inoculum level significantly ($P<0.001$) influenced mean 43- and 89-day scores, with the lowest scores obtained for uninoculated controls (Tables 2 and 3).

4.2 Influence of Storage Temperature

Analysis of variance showed that storage temperature had significant effect ($P<0.001$) on scores at day 43 and 89. In addition, with packs carrying high inoculum analysis of variance revealed that storage temperature significantly influenced the mean times to the onset of blowing. The longest times to the onset of blowing (>99 days) were obtained for water treated uninoculated packs stored at -1.5 and 0°C (Table 1).

4.3 Influence of Treatment

Storage profiles showing the effect of treatment type on the onset of gas production in vacuum-packed chilled venison are presented in Figure 3.

Statistical analysis indicated that, at primary interaction level, treatment type had no significant effect on the mean time to the onset of gas production in packs carrying high inoculum. However, the significant effect of treatment was observed with secondary level interactions. With packs carrying high inoculum spores, Inspex^{200}-treated samples produced longer ($P<0.05$) mean times to the onset of 'blown pack' spoilage than water-rinsed samples during storage at -1.5°C. However, no significant effects were observed between treatment types with packs stored at 0 and 2°C.

Statistical analysis indicated that rinse treatment had statistically significant ($P<0.001$) effect on pack distension scores at the variate days 43 and 89.

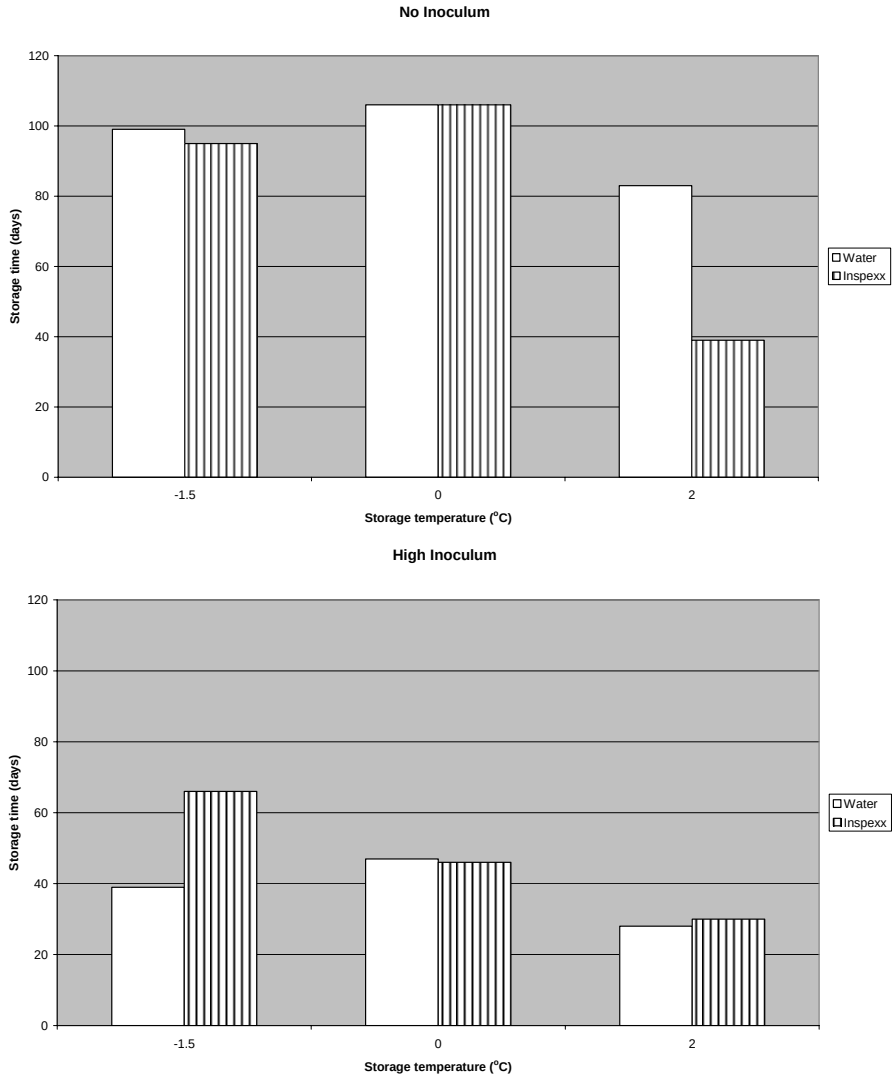

Figure 3. The influence of inoculum level, storage temperature and treatment type on mean time (in days) to the onset of 'blown pack' spoilage in vacuum-packed venison cuts. The data are means of five replicates. Water treatment is marked with solid white bars, and Inspexx^{200} treatment is marked with vertical stripes.

4.4 Molecular detection of *Cl. estertheticum*

The results of PCR detection of *Cl. estertheticum* in the exudates of vacuum-packed venison samples are shown in Table 4. PCR products of the expected size (approx. 230 bp) were amplified from a representative number of DNA templates from exudates of all packs carrying high inoculum and with DNA of *Cl. estertheticum* DSM 8809^{T}. No PCR products were obtained with DNA templates from exudates of uninoculated control packs with which pack distension scores never exceeded 1, or with reagent-only blanks. However, PCR products of the expected size were confirmed with uninoculated controls in which pack distension scores exceeded 1 by day 106 of the trial.

Table 4. Molecular detection of *Cl. estertheticum* in exudates from vacuum-packed venison stored for 106 days at -1.5, 0 or 2°C. Exudates were collected from packs representing each treatment/inoculum/storage temperature combination

Storage temperature (°C)	Inoculum level	Treatment	Pack distension score at day 106	PCR amplification of *Cl. estertheticum*
-1.5	none	water	1	-
-1.5	none	water	0	-
-1.5	none	water	3	+
-1.5	high	water	3	+
-1.5	none	Inspexx^{200}	0	-
-1.5	none	Inspexx^{200}	4	+
-1.5	high	Inspexx^{200}	1	+
-1.5	high	Inspexx^{200}	4	+
-1.5	high	Inspexx^{200}	3	+
0	none	water	0	-
0	none	water	0	-
0	high	water	4	+
0	none	Inspexx^{200}	0	-
0	none	Inspexx^{200}	1	-
0	high	Inspexx^{200}	5	+
2	none	water	1	-
2	none	water	3	+ (weak)
2	high	water	4	+ (weak)
2	none	Inspexx^{200}	4	+
2	none	Inspexx^{200}	3	+
2	high	Inspexx^{200}	4	+

+ = positive PCR amplification, - = negative PCR amplification

5. Discussion

Natural variability of fresh meat model and unavoidable over handling of experimental meat samples which may occur in multivariate trials can have a significant effect on background contamination levels. These differences in turn occasionally contribute to, or cause, the high variance within replicates which may exaggerate or conceal treatment differences. In the present study, the lack of positive amplification from meat exudates of all but 2 uninoculated control packs stored at 0°C and below indicates that *Cl. estertheticum* spores that could naturally occur on experimental venison were either absent or present in numbers below the detection limit of the PCR assay employed. Combined with the absence of blowing for these uninoculated control packs the results of this study indicate that at storage temperatures 0 and -1.5°C, naturally occurring *Cl. estertheticum* spores were unlikely to have a significant effect on the observed differences in

pack blowing. Consequently, within the experimental design employed, the trial variables had a discernable influence on the time to, and extent of, 'blown pack' spoilage.

Inoculum level which simulates first carcass, and then sub-primal cut, contamination with clostridial spores is a major determinant of the number of spores that survive rinse treatments and become activated by heat shrinking during the packaging process. The results of the present study demonstrate the dramatic detrimental effect of inoculum on the onset of 'blown pack' spoilage of vacuum-packed chilled venison. In this study, the presence of 260 spores per cm^2 effectively decreased by two thirds the nominal shelf life of vacuum-packed venison. The results of this study indicate that dressing hygiene, and especially the hygiene of the carcass opening cuts and pelt removal, is of paramount importance for maximising storage life with respect to clostridial 'blown pack' spoilage.

Storage temperature is arguably the most important variable influencing the growth of microorganisms that survive a packaging process. The results of the present study demonstrate the significant effect raising the storage temperature above 0°C has on the time to gas production in vacuum-packed chilled venison. At the inoculum level employed in the present study, the onset of blowing can be delayed by at least 11 days by storing the product at 0°C and below rather than at 2°C. Therefore, to maximise storage life with respect to clostridial 'blown pack' spoilage the importance of maintaining storage temperatures at or below 0°C cannot be over emphasised.

In the present study treatment with Inspexx^{200} delayed the onset of pack blowing in packs carrying high inoculum and subsequently stored and -1.5°C, but not in packs stored at 0 and 2°C. Immediate reason for this effect is unknown. However, it is possible that the obtained delay to the onset of pack blowing is a result of a combined 'hurdles' effect of both treatment and reduced storage temperature (Leistner 1992; McMeekin 2000).

In the previous study *in vitro* inactivation of approx. 4 to 5 log cfu ml^{-1} *Cl. estertheticum* spores was readily obtained with peroxyacetic acid-based agent, Inspexx^{200} (Broda 2006). This level of spore inactivation was maintained in the presence of animal fat. In the present study, treatment with Inspexx^{200} did not result in inactivation of spores as indicated by subsequent blowing of packs carrying *Cl. estertheticum* inoculum. The disappointing, while not entirely surprising, result of this study is likely due to the attachment of clostridial spores to the connective/fat tissues of dressed carcasses. Spores are highly hydrophobic

(Rönner *et al*. 1990) and attach to food surfaces at a greater rate than vegetative cells (Husmark and Rönner 1992). It is recognised that attachment to surfaces affects bacterial resistance to sanitizers (Bower *et al*. 1996) and the dressed carcass surface may influence their efficacy by physically hindering sanitizer's approach to an attached cell. In contrast, microorganisms that are freely suspended in aqueous solutions are fully susceptible to the cidal action of sanitizers.

In practical terms the results of the present study indicate that the Inspexx^{200} treatment trialled will not eliminate the spoilage threat posed by clostridial 'blown pack' spoilage spores present on meat surfaces. However, the present study points strongly to the adoption of "best practices" to delay or prevent the onset of 'blown pack' spoilage within a reasonable commercial chilled storage period. Clearly a best practice would include the use of stringent process hygiene at dressing and pelt removal to minimise the initial contamination of carcass meat with spores of 'blown pack'-causing clostridia. Following packaging, the temperature of the product should be reduced to -1.5°C and held at that temperature to as close to the point of retail sale/end use as is practicable. When combined with final product storage temperature of -1.5°C, Inspexx^{200} rinse can be recommended for an extension of shelf-life of venison cuts contaminated with *Cl. estertheticum* spores.

6. Acknowledgements

The work in this study was conducted under contract to DEEResearch Limited.

7. References

Boerema J.A., Broda, D.M., and Bell, R.G. (2003) Abattoir sources of psychrophilic clostridia causing 'blown pack' spoilage of vacuum-packed chilled meats determined by culture-based and molecular detection procedures. *Letters in Applied Microbiology* **36**, 406-411.

Bower, C.K., McGuire, J. and Daeschel, M.A. (1996) The adhesion and detachment of bacteria and spores on food-contact surfaces. *Trends in Food Science & Technology* **7**, 152-157.

Broda, D.M., Bell, R.G., Boerema J.A. and Musgrave, D.R. (2002) The abattoir source of culturable psychrophilic *Clostridium* spp. causing 'blown pack' spoilage of vacuum-packed chilled venison. *Journal of Applied Microbiology* **93**, 817–824.

Broda, D.M., J.A. Boerema and R.G. Bell. (2003). PCR detection of psychrophilic *Clostridium* spp. causing 'blown pack' spoilage of vacuum-packed chilled meats. *Journal of Applied Microbiology* **94**: 515-522.

Broda, D.M. (2006) The effect of peroxyacetic acid-based sanitizer, heat and ultrasonic waves on the survival of *Clostridium estertheticum* spores *in vitro. Letters in Applied Microbiology* (submitted)

Husmark, U. and Rönner, U. (1992) The influence of hydrophobic electrostatic and morphologic properties on the adhesion of *Bacillus* spores. *Biofouling* **5**, 335-344.

Iturriaga, M.H., Escartin, E.F., Beuchat, L.R. and Martínez-Peniche, R. (2003) Effect of inoculum size, relative humidity, storage temperature, and ripening stage on the attachment of *Salmonella* montevideo to tomatoes and tomatillos. *Journal of Food Protection* **66**, 1756-1761.

Leistner L. (1992) Food preservation by combined methods. *Food Research International* **25** 151-158

Lund, B.M., Graham, A.F., George, S.M. and Brown, D. (1990) The combined effect of incubation temperature, pH and sorbic acid on the probability of growth of non-proteolytic type B *Clostridium botulinum*. *Journal of Applied Bacteriology* **69**, 481-492.

McMeekin, T.A., Presser K., Ratkowsky D., Ross T., Salter M. and Tienungoon S. (2000) Quantifying the hurdle concept by modelling the bacterial growth/no growth interface. *International Journal of Food Microbiology* **55**, 93-98

Rönner, U., Husmark, U. and Henriksson, A. (1990) Adhesion of *Bacillus* spores in relation to hydrophobicity, *Journal of Applied Bacteriology* **69**, 550-556.

Takeuchi, K. and Frank, J.F. (2000) Penetration of *Escherichia coli* O157:H7 into lettuce tissues as affected by inoculum size and temperature and the effect of chlorine treatment on cell viability. *Journal of Food Protection* **63**, 434-440.