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Investigation into Agents Suitable for the Removal or Inactivation of Spores of "Blown Pack" Causing *Clostridium* Species from the Pelts of Live Deer

July 2007

CR 1188

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Investigation into Agents Suitable for the Removal or Inactivation of Spores of "Blown Pack" Causing *Clostridium* Species from the Pelts of Live Deer

Prepared for DEEResearch

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July 2007

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Summary

Two experiments were carried out to compare the efficacy of a selection of sanitisers for inactivation of *Clostridium estertheticum* spores *in vitro*. For the first experiment *C. estertheticum* spores were exposed to various concentrations of a peroxyacetic acid – based carcass wash (Ecolab Inspexx), a commercial live animal washing solution containing a mixture of non-ionic and anionic surfactants with a quaternary ammonium compound (Klenzion Agwash), another commercial live animal washing solution containing stabilised glutaraldehyde and surfactants (Ecolab Stockwash), and a sodium hypochlorite solution (Ecolab XY-12). Treatments with sodium hypochlorite and Ecolab Inspexx at 50 ppm FAC and 180 ppm, respectively, resulted in the inactivation of at least 3.88 log CFU/ml of *C. estertheticum* spores. However, no reductions in spore numbers were observed in the samples treated with Ecolab Stockwash at all of the concentrations tested, and Klenzion Agwash at the concentrations 0.2:40 and 0.1:40, in comparison to the control treatment (peptone buffered saline wash). The second experiment investigated the efficacy of the above sanitisers and water for inactivating *C. estertheticum* spores on a deer pelt model. Treatment with water resulted in a reduction of 1.23 log CFU/cm_2 in comparison with the inoculated control. A reduction of 2.09 log CFU/cm_2 was achieved with Ecolab Stockwash in comparison with the inoculated control. No reductions in spore recovery were observed from treatments with Ecolab Inspexx, sodium hypochlorite and Klenzion Agwash in comparison with the water treatment. The study demonstrated that a livestock wash with Ecolab Stockwash is capable of removing and/or inactivating at least 2 log CFU/ml *C. estertheticum* spores on the deer pelt. It is believed that when used in combination with other measures to control "blown pack"-causing clostridia, this wash has the potential to considerably reduce the amount of clostridial spores transferred onto dressed carcasses. Consequently, the use of Ecolab Stockwash offers the opportunity to reduce the incidence of "blown pack" spoilage.

Contents

Page

Summary ..... **i**
Contents ..... **ii**

1. Introduction **1**
Rationale 1
Stabilised Glutaraldehyde 1
The Klenzion™ Agwash Stock Washing System 2
Sodium Hypochlorite 3
Electrolysed Oxidizing Water 3
Chlorine Dioxide 4

2. Objectives **4**

3. Materials and Methods **5**
3.1 Spore Preparation and Enumeration 5
3.2 *In vitro* Experiment 5
Experimental Treatments 5
Enumeration of Treated Spores 6
3.3 Deer Pelt Model Experiment 6
Deer Pelt 6
Inoculation of Deer Pelt 6
Experimental Treatments 7
Sampling and Enumeration 7
Identification of bacterial colonies with PCR 8

4. Results **8**
Enumeration of Spore Preparation 8
4.1 *In vitro* Experiment 8
Enumeration of Treated Spores 8
4.2 Deer Pelt Model Experiment 9
Enumeration of Treated Spores 9
Confirmation of Identification with PCR 10

5. Discussion **11**

6. Conclusion **13**

7. References **13**

1. Introduction

Rationale

"Blown pack" spoilage is caused by psychrophilic *Clostridium* spp., spore forming and obligately anaerobic bacteria that grow at refrigeration temperatures. Slaughter animals are the primary sources of these bacteria in a meat processing plant. Previous research has established that spores of clostridia capable of causing "blown pack" spoilage are commonly carried on pelts and in faeces of bovine, ovine and deer slaughter stock (Broda *et al*. 2002). It is believed that under conditions of hygienic meat processing, contamination of carcasses with these microorganisms occurs with spores that are present in soil particles and faecal material attached to the pelts of slaughter animals during carcass dressing (Broda *et al*. 2002, Boerema *et al*. 2003). Consequently, it is likely that control measures applied prior to carcass dressing could have the greatest impact on the onset and severity of "blown pack" spoilage, because they could prevent the contamination of dressed carcasses and meat processing surfaces further down the processing chain.

Studies have demonstrated that washing the hides of live cattle with antimicrobial agents such as cetylpyridinium chloride or sodium hydroxide reduces microbial contamination of the hide and may result in improved carcass microbiology (Bosilevac *et al.* 2004, Bosilevac *et al*. 2005). These studies did not however investigate the effects of hide washing on the carriage of clostridial spores. Pre-slaughter washing with sporicidal or spore-removing agents may be a means for reducing contamination of carcasses with *Clostridium* spp. capable of causing "blown pack" spoilage.

An agent that is suitable for use as a live animal wash to reduce the amount of "blown pack" causing clostridia transferred from pelts to carcasses during slaughter must have sporicidal or spore-removing properties. However, the agent must not cause harm to live animals. A selection of agents with potential for use in live animal washes to kill or remove the spores of "blown pack" causing *Clostridium* spp is listed below:

Stabilised Glutaraldehyde

Glutaraldehyde and formaldehyde are the two most commonly used aldehydes with sporicidal action (Broda 2004). For glutaraldehyde to be an effective cleaning, disinfecting or sterilizing agent, however, the pH of the glutaraldehyde solution must be in the range of 7 to 8.5. It is difficult to maintain glutaraldehyde solutions at this pH as they are unstable (Martin 1997). Various stabilisers are currently added to glutaraldehyde solutions by the operator just before use to bring the pH into the optimally active range. In the presence of these stabilizers glutaraldehyde solutions have a shelf life of two to four weeks at a pH of 7-8.5 (Cleanroom Technology 2006). There are also a number of safety issues surrounding

glutaraldehyde to be considered such as skin irritation, corrosion, sensitisation and potential respiratory problems (NIOSH 2001).

Stabilised glutaraldehyde (G-Cide) is a combination of glutaraldehyde and a non-toxic, stabilizing surfactant molecule with which glutaraldehyde complexes. The formulation process results in glutaraldehyde molecules having predominately linear chain structures, which is the most active form. G-cide was developed to incorporate the well documented antimicrobial activity of glutaraldehyde without the side effects of this unstable, volatile molecule (Perks 2005). G-Cide is non-corrosive, non-volatile, non-toxic, biodegradable, stable, and highly antimicrobial at neutral pH (Lambrechts *et al*. 2004).

Ecolab markets a commercial live animal wash in New Zealand containing G-cide under the name ECOSAFE ^{TM} Stockwash Plus. This product is an antimicrobial, pH neutral wash, designed to aid in the removal of soil and reduce the numbers of microorganisms on the hides of animals prior to slaughter (Ecolab 2002).

The Klenzion™ Agwash Stock Washing System

Klenzion Ltd, in association with its chemical supplier, Colourlock Industries Ltd developed a product known as Agwash™ to reduce visual and microbial contamination of livestock prior to slaughter. Klenzion has also developed equipment for the application of Agwash in order to clean animals prior to slaughter. The system involves passing livestock through an automatic applicator where Agwash detergent sanitizer is applied as a spray. Livestock are then contained within a holding pen for 10 minutes to allow the Agwash solution to act on hide contaminants. Livestock are then passed through the Klenzion Restrainer Washing Unit (sheep) or an existing plant washing system (cattle) for a potable water rinse to remove the organic loading. If necessary, livestock can be sprayed with AgSan™ to minimize any remaining residual microbial loadings. As stock must be "substantially dry" prior to being presented to the slaughter floor Klenzion can also supply a patented drying system for sheep that reduces the drying time prior to slaughter and helps to minimize the risk of recontamination (Klenzion Ltd 2006).

The patent for the detergent sanitizer application apparatus specifies that a suitable cleaning fluid will assist in releasing dirt from the animal, will “wet” the animal and remain on the wool or hide of the animal for the required holding period, will not taint the meat if it comes into contact with the incision, and will not harm or stain the pelt. Also, the detergent should be approved for use by appropriate regulatory authorities. The cleaning fluid recommended in the patent is a mixture of non-ionic and anionic surfactants having an approximate pH of 11. The cleaning fluid can also include a bacteriocide. According to the patent a suitable bacteriocide for use with the apparatus is a quaternary ammonium compound, such as ACTICIDE which is available from Thor Chemicals Ltd

(Ramsey & Jones 2000). Quaternary ammonium compounds are not sporicidal. However, the Klenzion™ Agwash Stock Washing System may be able to reduce the number of 'blown pack' causing clostridia spores present on deer pelts by physically removing spores and spore-containing organic material. This would potentially mean a lower transfer rate from pelt to carcass during slaughter and dressing.

Sodium Hypochlorite

A 10^4 reduction in the recovery of *Bacillus stearothermophilus* spores was achieved in 9.4 minutes with a 0.05% solution of sodium hypochlorite at pH 7.0, but the same effect was achieved in 3.5 minutes when the spores were treated with a 0.1% solution (Mazzola *et al*. 2003). With *B. subtilis* spores, a 0.05% solution of sodium hypochlorite at pH 7.0 killed more than 10^4 in 30 min (Sagripanti & Bonifacino 1996). In a trial by Mies *et al* (2004) to determine the efficacy of cattle wash treatments in reducing pathogens on hides of cattle before slaughter, a sodium hypochlorite solution containing 50 ppm free available chlorine was applied to live cattle at a commercial beef processing facility in the United States of America.

The sporicidal action of sodium hypochlorite is reported to be reduced by the presence of organic matter. However, the application of a live animal wash containing sodium hypochlorite solution could be preceded by measures such as high pressure water rinsing or application of chemical surfactants to reduce the load of organic material on the pelts of slaughter animals.

Electrolysed Oxidizing Water

In order to produce electrolysed oxidizing (EO) water a current of electricity is passed through a dilute NaCl solution inside a chamber where the anode and cathode electrodes are separated by a charged bipolar membrane. Basic EO water containing sodium hydroxide is produced at the cathode and has a strong reducing potential. Acidic EO water with a strong oxidising potential, containing hypochlorous acid and 10 to 100 ppm free chlorine is produced at the anode (Bach *et al.* 2006). Research by Ayebah *et al*. (2005) showed that EO water was relatively non-corrosive when applied to materials that are commonly used in the food industry. EO water is environmentally friendly and safe to handle as salt is the only chemical used (Midgley & Small, 2006).

EO water produced from a JAW-020 generator (Nippon Intek) reduced *B. cereus* spores from 6.89 log CFU/mL to 1.4 log CFU/ml in 120 seconds. After 120 seconds of treatment with EO water produced from a ROX-20TA generator (Hoshizaki Electric) *B. cereus* spores were reduced from 6.93 log CFU/ml to 3.44 log CFU/mL. These results were obtained from a study by Kim *et al.* (2000) that was undertaken to evaluate the efficacy of EO and chemically modified water with

properties similar to EO water for inactivation of different types of food borne pathogens.

Chlorine Dioxide

Beuchat *et al*. (2005) demonstrated that treatment of *B. cereus* and *B. thuringiensis* spores in a medium containing 3.4 mg/mL organic and inorganic solids with an equal volume of alkaline (pH 12.1) ClO_2 (400 mg/ml) for 30 minutes reduced populations by 4.6 and 5.2 log, respectively. This indicates that alkaline ClO_2 has a high lethality against the spores of these organisms in the presence of materials that would potentially react with and neutralise the sporicidal activity of ClO_2. Alkaline ClO_2 solutions were prepared using an electro-generator using patent-pending electrochemical technologies (Procter and Gamble, Cincinnati, Ohio). According to Beuchat *et al*. (2005) there could be potential in the use of alcohols in combination with ClO_2 to enhance lethality to *Bacillus* spores on the surface of, or within organic substrates and on hard inert surfaces, and this warrants further research.

Unfortunately chlorine dioxide releases fumes that irritate the respiratory tract and it corrodes some metals (BSG Endoscopy Committee Working Party 1998). Therefore it is probably not suitable for application to live animals in a meat processing environment. However, a patent exists for a skin cleanser that is suitable for application to sensitive areas of the skin of humans and animals, and contains chlorine dioxide in conjunction with other components (Alliger & Roozdar 1997). Depending on availability such a product, it may have potential for use on the pelts of live animals to remove or inactivate the spores of "blown pack" causing clostridia.

This study investigated the sporocidal activity of stock and carcass washes currently approved for use in NZ.

2. Objectives

The aim of the project was to develop a pre-slaughter wash regime suitable for use on live deer that will inactivate or remove spores of *Clostridium* spp. from pelts. This could result in reduced transfer of clostridial spores from pelts to carcasses during the processing of venison. Two experiments were carried out to compare the efficacy of a selection of sanitisers for inactivation of *Clostridium estertheticum* spores *in vitro*. For the first experiment *C. estertheticum* spores were exposed to various concentrations of a peroxyacetic acid – based carcass wash, a commercial live animal washing solution containing a mixture of non-ionic and anionic surfactants with a quaternary ammonium compound, another commercial live animal washing solution containing stabilised glutaraldehyde and surfactants, and a sodium hypochlorite solution. The second experiment

investigated the efficacy of these sanitisers and water at inactivating *C. estertheticum* spores on a deer pelt model.

3. Materials and Methods

3.1 Spore Preparation and Enumeration

C. estertheticum 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 Schott bottle was inoculated with an exponentially growing culture of *C. estertheticum* DSM 8809^T and incubated anaerobically at 10°C for a minimum of 6 months to promote sporulation.

Spores were harvested when a significant number of spores, as evidenced by the presence of refractive spheres, were observed with phase contrast microscopy. The broth culture was centrifuged, the supernatant was discarded and then the pellet was re-suspended in ice-cold physiological saline. The re-suspended spore suspension was heated to 60°C for 10 min to eliminate any remaining vegetative cells. The heat treated spore suspension was washed two more times with ice-cold sterile physiological saline and finally re-suspended in ice-cold saline. Spore suspensions were dispensed into eppendorf tubes and stored at -18°C prior to use. Since conditions in saline suspension do not induce activated spores to germinate, the spores revert to a dormant state during frozen storage.

The concentration of *C. estertheticum* spores used for these experiments was determined by a tenfold dilution series plated onto sterile Columbia Blood Agar plates (CBA, Oxoid), containing 5% sheep's blood. Inoculated plates were incubated anaerobically at 10°C for 3 weeks then enumerated.

3.2 *In vitro* Experiment

Experimental Treatments

Sanitising agents to be tested were each prepared at three dilutions with sterile distilled water; Klenzion Agwash – 1:40, 0.2:40 and 0.1:40, Ecolab Inspexx (peroxyacetic acid based carcass rinse) – 180 ppm, 36 ppm and 18 ppm, Ecolab Stockwash - 0.5:50, 0.1:50 and .05:50, and sodium hypochlorite (Ecolab XY-12) – 50 ppm free active chlorine (FAC), 10 ppm FAC and 5 ppm FAC. Aliquots (0.9 ml) of the diluted sanitising agents were dispensed into sterile eppendorf tubes. The concentration of FAC in the most concentrated sodium hypochlorite solution was confirmed to be 50 ppm with a titration kit supplied by manufacturer. The two remaining sodium hypochlorite solutions were prepared by diluting the solution that contained 50 ppm FAC.

Spore suspensions were prepared as a ten-fold dilution series from -1 to -6 with sterile distilled water. A volume (0.1 ml) of appropriately diluted spore suspension was added to each of the eppendorf tubes containing sanitising agents. All spore concentration and sanitizer dilution combinations were tested in duplicate. The tubes were mixed with a vortex mixer and incubated at room temperature for 5 min. Control tubes contained a volume of phosphate buffered saline (0.9% NaCl, pH 6.9) (PBS, Lorne Laboratories Ltd), to which spore suspensions were added.

Enumeration of Treated Spores

Immediately upon completion of the incubation period the tubes were centrifuged at 15000 rpm for 5 minutes, washed twice with sterile PBS and re-suspended in PBS. Each sample was then plated in duplicate onto CBA plates containing 5% sheep's blood. Inoculated plates were incubated anaerobically at 10°C for 21 days. 0.1 ml of appropriately diluted spore suspension was added to 0.9 ml of each sanitiser. The highest concentration of spore suspension used was -1. A volume (0.1 ml) of treated sample was plated onto CBA plates. Hence, the limit of detection for the *in vitro* trial was 1 x 10^3 CFU/ml.

3.3 Deer Pelt Model Experiment

Deer Pelt

A pelt was pulled from a healthy adult deer immediately after the animal had been slaughtered at a local venison processing plant. The pelt was rolled up in low density polyethylene wrap in order to minimise cross contamination between different regions of the pelt and transported on ice to the laboratory. The pelt was then transferred onto a sterile surface.

Whirl Pak bags (Nasco, USA) were cut open and attached to polystyrene boards covered with sheets of plastic so that the sterile internal surface of each Whirl Pak bag faced outwards. 10 x 10 cm pieces (approximate surface area 100 cm^2) of pelt were aseptically cut from the regions of the pelt that corresponded with the sides of the deer. Sterile nails were used to attach three pieces of pelt to each board in such a way that the sterile internal surfaces of the Whirl Pak bags formed sterile barriers between the skin side of each piece of pelt and the board.

Inoculation of Deer Pelt

Pelt samples designated for inoculation were each inoculated with a volume (0.5 ml) of 1 x 10^6 cfu/ml spore suspension evenly distributing to each piece of pelt to obtain a final load of 5 x 10^3 cfu/cm^2. A sterile spreader was used to thoroughly work the spore solution into the pelt of each sample. Pelt samples designated for no inoculum each had a volume (0.5 ml) of sterile physiological saline evenly distributed (uninoculated controls). All samples were left at room temperature for 10 minutes to allow the spore or physiological saline inoculum to absorb into the pelts and dry.

Experimental Treatments

For each treatment, a polystyrene board holding three inoculated pelt samples was secured to the wall of a spray cabinet. Triplicate inoculated pelt pieces were subjected to treatment with the following sanitising agents: water, Ecolab Inspexx, sodium hypochlorite, Klenzion Agwash and Ecolab Stockwash. A set of three inoculated pieces of pelt were not treated with any of the sanitising agents (inoculated controls).

The spray applicator consisted of 3 spray nozzles located 28 cm apart on a rotating delivery tube. A Wilden pump with a pulse dampener was set to deliver an even pressure of 70 psi at each spray nozzle. A single row of 3 samples placed at the level of the central nozzle was sprayed each time. The distance between the spray nozzles and each sample was 70 cm. The volume of liquid applied to each set of 3 samples was 6 L. All of the treatments tested were left in contact with the pieces of pelt for 10 minutes before being rinsed with potable water following the same procedure used to apply the pelt washing agents. The residues of pelt washing agents were flushed out of the spray applicator with potable water before the application of each water rinse.

Triplicate samples allocated for treatment with water were sprayed first using potable water. Klenzion Agwash, Ecolab Stockwash, Ecolab Inspexx and sodium hypochlorite (XY-12, Ecolab) were diluted in potable water to achieve final concentrations of 1:40, 1%, 180 ppm and 50 ppm free available chlorine (FAC), respectively according to manufacturers' instructions. The concentration of FAC in the sodium hypochlorite solution was confirmed with a titration kit supplied by manufacturer.

After the final water rinse for each triplicate the pieces of pelt were aseptically transferred into sterile 1.6 L Whirl Pak bags. A set of three uninoculated pieces of pelt were also aseptically transferred into sterile 1.6 L Whirl Pak bags (uninoculated controls). The bags were left open for 30 minutes to allow the samples to dry slightly. The bags were then sealed and stored overnight at 2°C.

Sampling and Enumeration

Ice-cold physiological saline (400 ml) was added to each Whirl Pak bag. Each bag was stomached for 2 minutes and the stomached supernatant was transferred to sterile centrifuge bottles. The samples were centrifuged at 4500 rpm for 20 minutes.

The supernatant was discarded and the pellets were re-suspended in 10 ml ice-cold physiological saline. The samples were then transferred into sterile test tubes and left to settle for 1 minute to allow some of the crude material such as hairs and dirt to settle to the bottom of the tubes. The samples were then transferred to sterile centrifuge tubes, transferring as little of the crude material as possible. The

samples were heated to 60°C for 10 minutes. Thermometer was placed into a pilot tube containing physiological saline in order to monitor the heating process. The heat treated samples were centrifuged at 15000 rpm for 5 minutes. The centrifuge was set to maintain an internal temperature of 5°C during all centrifugation cycles.

The supernatant was discarded and the pellets were re-suspended in 2 ml ice-cold physiological saline and numbers of *C. estertheticum* spores present in the pellet from each sample were determined by a tenfold dilution series plated onto the surface of freshly prepared Columbia Blood Agar plates (CBA, Oxoid), containing 5% (v/v) sterile sheep's blood. Inoculated plates were incubated anaerobically at 10°C for 3 weeks then enumerated by counting the colonies that resembled those of *C. estertheticum*, evidenced by grey, slightly translucent colonies that demonstrated haemolysis.

Identification of bacterial colonies with PCR

In order to confirm that *C. estertheticum* colonies were being identified correctly, DNA was extracted from triplicate representative colonies from each treatment type and subjected to PCR with primers specific for the 16s ribosomal DNA of *C. estertheticum* (Broda *et al*. 2003). The expected size of the PCR product was 790 base pairs. PCR products were subjected to gel electrophoresis on a 1.5% agarose gel.

4. Results

Enumeration of Spore Preparation

The concentration of *C. estertheticum* spores in the spore preparation used for these experiments was 7.5 x 10^6 CFU/ml.

4.1 *In vitro* Experiment

Enumeration of Treated Spores

The results of the experiment are shown in Figure 1. Number of spores recovered from samples treated with PBS only (the control treatment) was 6.28 log CFU/ml. The recovery of spores from the samples that did not undergo any treatment was 6.88 log CFU/ml. No reductions in spore numbers were observed in the samples treated with Ecolab Stockwash at all of the concentrations tested, and Klenzion Agwash at the concentrations 0.2:40 and 0.1:40, in comparison to the control treatment (PBS). Numbers of spores recovered from samples that underwent treatment with Klenzion Agwash at a concentration of 1.0:40 were by 0.26 log CFU/ml lower than those recovered in control treatment (PBS). No colonies were detected for the samples treated with sodium hypochlorite (Ecolab XY-12) and Ecolab Inspexx at 50 ppm FAC and 180 ppm respectively. Spore recoveries of 4.11 and 4.33 log CFU/ml were obtained from samples treated with sodium

hypochlorite at 10 and 5 ppm FAC, respectively. Spore recoveries of 5.04 and 5.41 log CFU/ml were obtained from samples treated with Ecolab Inspexx at 36 and 18 ppm, respectively.

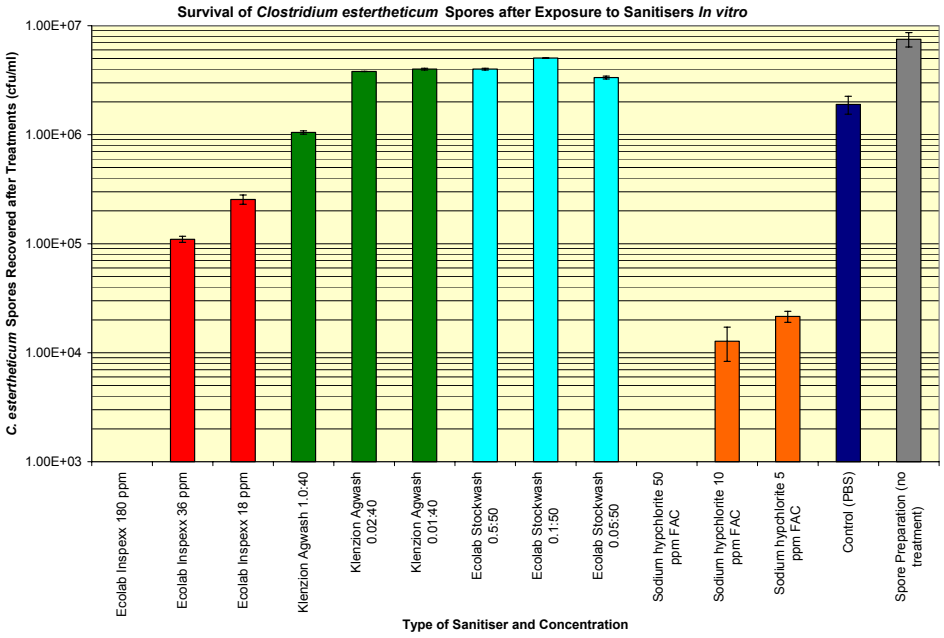

Figure 1. Survival of *C. estertheticum* spores following treatments with various sanitising agents *in vitro*.

4.2 Deer Pelt Model Experiment

Enumeration of Treated Spores

The results obtained in the experiment are illustrated in Figure 2. Spore recovery from the inoculated pieces of deer pelt that did not undergo any treatment (inoculated control) was 3.74 log CFU/cm^2. Treatment with water resulted in a reduction of 1.23 log CFU/cm^2 in comparison with the inoculated control. A reduction of 2.09 log CFU/cm^2 was achieved with Ecolab Stockwash in comparison with the inoculated control. No reductions in spore numbers were observed from treatments with Ecolab Inspexx, sodium hypochlorite and Klenzion Agwash in comparison with the water treatment.

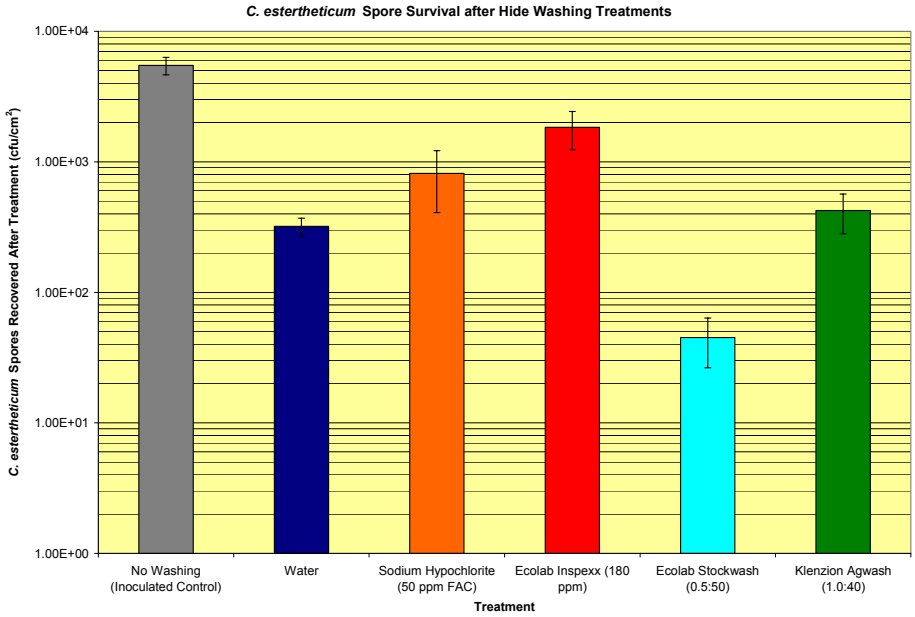

Figure 2. Survival of *C. estertheticum* spores following treatments with various sanitising agents on a deer pelt model.

Confirmation of Identification with PCR

Figure 3 is an image of the agarose gel which demonstrates the results from the PCR confirmation assay. Refer to Table 1 for the layout of samples loaded onto the gel. Samples 1-15 were from the treatment types in triplicate while samples 16-18 were from the inoculated control samples. All of these samples gave a positive PCR reaction of the expected size except sample 13. Samples 19 – 26 were taken from uninoculated control plates. These samples represent any colonies that slightly resembled *C. estertheticum* on the plates from the uninoculated pelt samples. No bands were visible in the lanes loaded with samples 19 – 26, indicating that there was no natural contamination with *C. estertheticum* on the pieces of deer pelt used for this experiment.

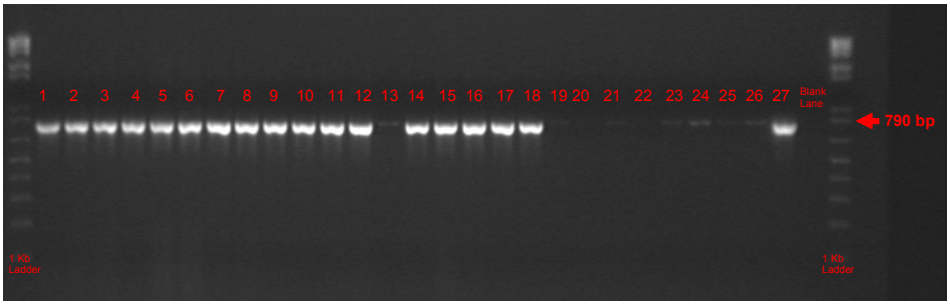

Figure 3. Image of agarose gel from PCR performed to confirm the identification of colonies that resembled *C estertheticum*

Table 1. Layout of PCR Samples

Sample #	Treatment	Plate
1	Ecolab Stockwash	A
2	Ecolab Stockwash	B
3	Ecolab Stockwash	C
4	Ecolab Inspexx	A
5	Ecolab Inspexx	B
6	Ecolab Inspexx	C
7	Water	A
8	Water	B
9	Water	C
10	Sodium hypochlorite	A
11	Sodium hypochlorite	B
12	Sodium hypochlorite	C
13	Klenzion Agwash	A
14	Klenzion Agwash	B
15	Klenzion Agwash	C
16	Inoculated control	A
17	Inoculated control	B
18	Inoculated control	C
19	Uninoculated control	A1
20	Uninoculated control	A2
21	Uninoculated control	B1
22	Uninoculated control	B2
23	Uninoculated control	C1
24	Uninoculated control	C2
25	Uninoculated control	C3
26	Uninoculated control	C4
27	Positive control	

5. Discussion

The aim of this project was to develop a pre-slaughter wash regime suitable for use on live deer that would inactivate or remove spores of *C. estertheticum* from pelts. Ecolab Stockwash was the only treatment that was more effective than water at reducing the recovery of *C. estertheticum* spores from the deer pelt model. A reduction of 2.09 log cfu/cm^2 was achieved with Ecolab Stockwash in comparison with the inoculated control. Treatment with Ecolab Stockwash resulted in a reduction in spore recovery of 0.86 log cfu/cm^2 when compared to the water treatment.

It is believed that under conditions of hygienic meat processing, contamination of carcasses with spores of *Clostridium* spp. capable of causing "blown pack" spoilage may occur during dressing when spores that are present in soil particles and faecal material attached to the pelts of slaughter animals come in contact with the carcass (Broda *et al*. 2002, Boerema *et al*. 2003). Although the 2.09 log cfu/cm^2 reduction in spore recovery achieved with Ecolab Stockwash on the deer pelt model may not initially appear to be major, this reduction, in combination with other measures used to control "blown pack" causing clostridia (e.g. improved dressing hygiene, storage of the vacuum-packed product at -1.5°C) could have the potential to considerably reduce the amount of clostridial spores transferred onto carcasses, as well as severity and frequency of pack blowing. This could reduce the incidence of "blown pack" spoilage.

Major reductions in spore recovery were observed in the *in vitro* trial with sodium hypochlorite (Ecolab XY-12) and Ecolab Inspexx at 50 ppm FAC and 180 ppm respectively. However, treatments with these agents in the deer pelt model trial did not result in reductions in spore recovery in comparison with water, Ecolab Stockwash and Klenzion Agwash. This suggests that the sporicidal activity of Ecolab Inspexx and sodium hypochlorite was inactivated under the experimental conditions employed in the deer pelt model trial. Previous research has shown that organic material may consume available chlorine (Environmental Health and Safety, 2007). Gehr *et al.* (2003) reported that the antimicrobial effectiveness of peroxyacetic acid is also reduced in the presence of the organic material. Organic material such as dirt, proteins and natural oils would have been present on the deer pelt used for this trial. It is possible that the concentrations of sodium hypochlorite and Ecolab Inspexx applied to the pelt model were insufficient to overcome the reduction in antimicrobial activity caused by the presence of this organic matter.

The *in vitro* trial investigated the sporicidal properties of selected sanitising and/or cleaning agents, and showed that survival of *C. estertheticum* spores can be reduced in the presence of Ecolab Inspexx and sodium hypochlorite when these agents are applied to spores suspended in liquid. In the deer pelt model used in the present study spores were present in, or perhaps attached to, a semi-solid matrix of various pelt constituents, The reductions in spore recoveries achieved in the deer pelt model trial could have occurred as a result of either sporicidal activity or physical removal of spores due to the washing effect of the spray application. Treatments with Ecolab Stockwash did not achieve major reductions in spore recovery in the *in vitro* trial. Conversely, Ecolab Stockwash was the most effective treatment on the deer pelt model. Hence, it is likely that the reduction in spore recovery observed with Ecolab Stockwash on the deer pelt model was a result of physical spore removal.

6. Conclusion

Under the experimental conditions employed in the present study Ecolab Stockwash is capable of removing and/or inactivating at least 2 log CFU/ml *C. estertheticum* spores on the deer pelt. It is believed that when used in combination with other measures to control "blown pack"-causing clostridia, this wash has the potential to considerably reduce the amount of clostridial spores transferred onto dressed carcasses. Consequently, the use of Ecolab Stockwash offers the opportunity to reduce the incidence of "blown pack" spoilage.

7. References

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