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Thermal inactivation of *Yersinia enterocolitica* in pork slaughter plant scald tank water

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ABSTRACT

The objective of this study was to establish the time–temperature combinations required to ensure the thermal inactivation of *Yersinia enterocolitica* during scalding of pork carcasses. A 2 strain cocktail of *Y. enterocolitica* (bioserotypes 2/O:5,27 and 1A/O:6,30) was heat treated at 50, 55 and 60 °C in samples of scald tank water obtained from a commercial pork slaughter plant. Samples were removed at regular intervals and surviving cells enumerated using (i) Cefsulodin–Irgasan–Novobiocin Agar (CIN) supplemented with ampicillin and arabinose and (ii) Tryptone Soya Agar (TSA), overlaid with CIN agar with ampicillin and arabinose. The data generated was used to estimate *D*- and *z*-values and the formula $D_x = \log^{-1}(\log D_{60} - ((t_2 - t_1)/z))$ was applied to calculate thermal death time–temperature combinations from 55 to 65 °C. D_{50}, D_{55} and D_{60}-values of 45.9, 10.6 and 2.7 min were calculated from the cell counts obtained on CIN agar, respectively. The corresponding *D*-values calculated from the TSA/CIN counts were 45.1, 11 and 2.5 min, respectively. The *z*-value was 7.8. It was concluded that a time–temperature combination of 2.7 min at 60 °C is required to achieve a 1 log reduction in *Y. enterocolitica* in pork scald tank water. The predicted equivalent at 65 °C was 0.6 min. This study provides data and a model to enable pork processors to identify and apply parameters to limit the risk of carcass cross-contamination with *Y. enterocolitica* in pork carcass scald tanks.

1. Introduction

In recent years, *Y. enterocolitica* has been the third most common cause of food borne disease after *Campylobacter* spp. and *Salmonella* spp. in Europe with 7,595 cases reported in 2009 (EFSA (European Food Safety Authority), 2011). The most common manifestation of *Y. enterocolitica* infection is gastro-enteritis which is usually self-limiting resulting in diarrhoea with mild fever and abdominal pain. The patient may also suffer nausea and vomiting. Occasionally, the infection may be restricted to the lymphatic tissue on the right side of the body resulting in symptoms that are often confused with appendicitis. Arthritic joint and skin complaints may be a further complication, even in patients with no previous gastrointestinal symptoms.

Pigs are the major source of *Yersinia enterocolitica* infection worldwide (Fredriksson-Ahomaa, Stolle, Siitonen, & Koekeala, 2006). Newborn piglets are easily colonised by *Y. enterocolitica* and become long-term carriers of the bacterium in the oral cavity and intestines (Schiemann, 1989). As a result, pigs are a primary reservoir of *Y. enterocolitica* and pork products have been implicated in many foodborne cases/outbreaks (EFSA (European Food Safety Authority), 2011; Johannessen, Kapperud, & Kruse, 2000). Porcine tonsils are frequently contaminated with this pathogen and contamination rates of 31–38% (Asplund, Tuovinen, Veijalainen, & Hirn, 1990), 37.3% (Van Damme, Habib, & De Zeutter, 2010) and 42% (De Boer & Nouws, 1991) have been previously reported. Approximately 80% of porcine tongues and oral cavities in general also carry *Y. enterocolitica* (Fredriksson-Ahomaa, Hielm, & Korkeala, 1999; Nesbakken, 1988; Nesbakken, Nerbrink, Rotterud, & Borch, 1994).

During pig slaughter and carcass processing a range of heating, washing and abrasive treatments are applied. As part of the dehairing process, pig carcasses are initially pulled through a large vat containing water at temperatures of approximately 57–62 °C, a process referred to as scalding which facilitates removal of the bristles from hair follicles in the subsequent stages. Many pigs may be scalded in the same scald tank and the water quickly becomes contaminated with faeces, dirt and ingesta. During the scalding process *Y. enterocolitica* are readily transferred from the oral cavity to the scald tank water. If these bacteria survive in the scald tank water, cross-contamination of subsequently scalded carcasses is inevitable. It is therefore essential to limit such contamination by manipulating the parameters involved in scalding to ensure the destruction of *Y. enterocolitica* cells entering the water without damaging the commercially important characteristics of the carcasses.

The objective of this study was to investigate the thermal inactivation of *Y. enterocolitica* in scald tank water and to provide clear guidance on the time–temperature combination required to eliminate the scald tank as a source of this pathogen during pork carcass processing.

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2. Materials and methods

2.1. Scald tank water

Samples of scald tank water were obtained from a commercial pig abattoir (approximately 900–1000 pigs slaughtered per day). Three 1 l samples were collected at intervals during the day's operations: (i) approximately 1 h after commencing slaughter, (ii) half way through slaughter (approx. 4 h into slaughter) and (iii) when slaughter operations had finished for the day (approx. 8 h into slaughter). Samples were maintained at approx 4 °C, transported to the laboratory and stored at approx. 2 °C overnight. Prior to use, the samples were pooled to obtain a sample representative of the mean status of the scald tank water during a typical day's production.

2.2. Bacterial cultures

A cocktail of two pork *Yersinia enterocolitica* strains was used in these experiments, *Y. enterocolitica*, biotype 2, serotype O:5, 27 (DSMZ 11504 obtained from the Deutsche Sammlung von Mikroorganismen Zellkulturen, Germany) and *Yersinia enterocolitica*, biotype 1A, serotype O:6, 30 (NCTC 11599 obtained from the National Collection of Type Cultures, UK). These bioserotypes were selected as recent studies suggest they are the most common bioserotypes isolated from human patients and pigs, respectively, in Ireland. To facilitate the recovery of these strains, both organisms were genetically modified by insertion of the plasmid, pGLO (Bio-rad Laboratories, California, USA) encoding for green fluorescent protein (GFP). This plasmid makes it possible to count colonies of the test bacteria in the presence of a large indigenous micro flora on media containing arabinose (0.6 mg ml^{-1}). The plasmid was inserted by transformation using heat shock in accordance with the pGLO Bacterial Transformation Kit instructions (Bio-rad Laboratories, California, USA). Briefly, 250 μl volumes of pGLO transformation solution (50 Mm CaCl2, Ph 6.1) were pipetted into sterile micro test tubes and transferred to an ice-bath (approximately 2 °C). A single colony of each *Y. enterocolitica* strain was aseptically immersed into the transformation solution and incubated in the ice-bath for 10 min. Tubes were transferred to a 42 °C water-bath for exactly 52 s then immediately returned to the ice-bath for 2 min. Tubes were placed at room temperature. 250 μl of Luria–Bertani Broth (LB Broth, Oxoid) was added to each tube and incubated at room temperature for 10 min. Samples of each tube were plated onto Luria–Bertani agar (LB agar, Oxoid). This was supplemented with ampicillin (0.1 mg ml^{-1}) and arabinose (0.6 mg ml^{-1}) which selected for the GFP transformed organisms and allowed them to be viewed under 365 nm UV light. The modified bacteria were stored on cryo-protective beads at −20 °C. Tests confirmed that these modified strains showed no significant difference in growth to their parent strains (data not shown).

2.2.1. Inoculum preparation

One bead of each modified *Y. enterocolitica* strain was aseptically transferred to 30 ml Brain Heart Infusion Broth (BHI, Oxoid) and incubated at 30 °C for 24 h. Following incubation a 1 ml aliquot from each culture was transferred to 100 ml BHI and incubated for a further 18 h at 30 °C. Each culture was then centrifuged at 3000 ×*g* for 10 min at 4 °C. The recovered pellet was washed three times with and resuspended in maximum recovery diluent (MRD). These suspensions were combined to form a cocktail. The cocktail had a final colony count of 10^8 CFU ml^{-1}.

2.3. Inoculation, sampling and enumeration

Volumes (90 ml) of scald tank water were dispensed into screw cap Medfor jars and equilibrated to 50, 55 and 60 °C by immersion in water baths at these temperatures, within 20 min. The temperature of the scald tank water samples was monitored using thermocouples inserted into 'blank' samples, and attached to a Squirrel SQ1600 Data Logger (Grant Instruments Ltd, Shepreth, UK). Immediately after equilibration, duplicate scald tank water samples were inoculated with 1 ml of the *Y. enterocolitica* cocktail. Samples of 10 ml were withdrawn at time zero (to check inoculation levels) and periodically thereafter, pipetted into a sterile thin-walled 30 ml plastic sterile bottle and immediately transferred to an ice-bath (approximately 2 °C).

Each cooled sample was serially diluted in MRD. Members of each dilution series were directly plated onto Cefsulodin–Irgasan–Novobiocin Agar (CIN agar, Oxoid) supplemented with ampicillin (0.1 mg ml^{-1}) and arabinose (0.6 mg ml^{-1}), and onto Tryptone Soya Agar (TSA, Oxoid). The CIN agar plates were incubated at 30 °C for 24 h. The TSA plates were incubated at 30 °C for 2 h, to allow recovery of injured cells, over-poured with CIN agar supplemented with ampicillin (0.1 mg ml^{-1}) and arabinose (0.6 mg ml^{-1}) (recovery overlay technique) and incubated for a further 24 h at 30 °C.

2.4. Calculation of D- and z-values and statistical analysis

Each experiment (50, 55 and 60 °C) was performed in duplicate (i.e. two inoculated bottles of scald tank water at each temperature) and each experiment was repeated three times using different samples of scald tank water. From the data, a plot of $\log_{10}$ of surviving cells ml^{-1} against time was prepared and the slope (b) and standard error (*S.E.*) were obtained for the plot at each temperature using linear regression analysis (Minitab, Minitab Inc., Pennsylvania, USA). *D*-values were calculated using the average slope for a given temperature treatment. Each *z*-value was calculated from the slope of an individual curve of a plot of $\log_{10}$ *D*-values against temperature by linear regression of the slopes of the plots. Thermal destruction times were calculated using the formula: $D_x = \log^{-1}(\log D_{60} - ((t_2 - t_1)/z))$ (Bolton, Pearce, Sheridan, McDowell, & Blair, 2003).

3. Results

The thermal death curves at the three treatment temperatures and the *D*-values are shown in Figs. 1–3 and Table 1. The D_{50} values were calculated as 45.87 min on CIN agar and 45.07 min using the TSA/CIN overlay technique. The corresponding D_{55} and D_{60} values were 10.55 and 2.67, respectively, on CIN agar and 10.98 and 2.53 min using the overlay technique. At each temperature the differences between the CIN and TSA/CIN based *D*-values were not significant ($P > 0.01$). The *z*-value obtained using CIN data was determined as 7.8 and using TSA/CIN counts as 7.9.

D-value predictions using the formula $D_x = \log^{-1}(\log D_{60} - ((t_2 - t_1)/z))$ (Bolton et al., 2003) are shown in Table 2. These suggest that the times required to achieve a 1 $\log_{10}$ CFU ml^{-1} reduction in *Y. enterocolitica* in scald tank water at 55, 56, 57, 58, 59, 60, 61, 62, 63, 64 and 65 °C are 10.98, 8.0, 6.0, 4.5, 3.37, 2.53, 1.88, 1.42, 1.07, 0.80 and 0.6 min, respectively.

4. Discussion

In this study D_{50} D_{55} and D_{60} values of 45.07, 10.98 and 2.53 min were determined experimentally and a range of *D*-values were predicted for temperatures ranging from 56 to 65 °C. Although there is very little thermal inactivation data for *Y. enterocolitica* published in the scientific literature, Pagan, Manas, Raso, and Trepat (1999) obtained D_{55} values of 0.33–0.78 min and D_{59} values of 0.18–0.6 min in citrate phosphate buffer. These values are considerable less than the equivalent values (10.98 and 3.37 min, respectively), reported in this paper.

A second study by Sorqvist and Danielsson-Tham (1990) reported D_{58}, D_{60} and D_{62} values of 1.3–1.8, 0.41–1.0 and 0.17–0.21 min, respectively. Again these values were multiple-fold less than the equivalent values of 4.5, 2.53 and 1.42 min, respectively, reported in our study.