Reduction of *Campylobacter* on Chicken Carcasses by SonoSteam® Treatment
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Reduction of *Campylobacter* on Chicken Carcasses by SonoSteam® Treatment

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Abstract

Contaminated food or water as well as poor cooking and kitchen hygiene are the typical causes of human *Campylobacter* infections. It is an increasing health problem and since poultry is the most common source, intervention programmes have been initiated to help eliminate the occurrence of *Campylobacter* on fresh poultry products. Attempts to decontaminate the surface of fresh meat by various methods have so far not been sufficiently effective. SonoSteam® is a new technology for decontamination of surfaces by the use of steam and ultrasound combined, and the method was tested on freshly slaughtered chickens in an off-line pilot plant, which was designed for the present investigations. Groups of 30 carcasses were included; one that was treated with SonoSteam® (sound-level of 160 dB and frequency of 20-25 kHz; steam at max. output of 100kg/h and temp. of 100° C) and another represented the references. All the carcasses were placed individually in sterile, airtight bags and analysed for the level of *Campylobacter* on the surface before and after SonoSteam® treatment. The results showed an average reduction of 2.51 $\log_{10}$ units (CFU/ml) and no visual changes of the chicken carcasses, which is as good as or better than currently used decontamination methods. A SonoSteam® in-line system is being developed and will be tested in the near future.

Keywords: Campylobacter, decontamination of surfaces, chicken, ultrasound, steam, zoonoses.

1. Introduction

Campylobacter jejuni is a microaerophilic and thermophilic gram-negative bacterium. It grows at a temperature between 30-45 °C – with optimum at 42°C (Cole *et al.* 2001, Ketley *et al.* 1997, Hansson *et al.* 2005, Jensen *et al.* 2006). It is widespread in nature and naturally occurring in the gastrointestinal tract of healthy warm blooded animals, especially birds (Willie *et al.* 1997, Berrang *et al.* 1999, Zhao *et al.* 2006, Altekruse *et al.* 1994). By presence in the faeces of production-animals, the bacteria might be transferred from the intestine to the fresh meat during slaughtering (Jeffrey *et al.* 2001, Willie *et al.* 1997, Hansson *et al.* 2005). This cross-contamination is particularly common within slaughter poultry but can also occur

amongst pigs and cattle. The bacteria are, however, sensitive to heat but can survive refrigeration (Sandberg *et al.* 2005, Wieland *et al.* 2006, Bhaduri *et al*. 2004).

Infection with *Campylobacter* is called campylobacteriosis and generates diarrhoea (occasionally bloody) (Jensen *et al.* 2006, Willie *et al*. 1997), abdominal pain, fever and sometimes nausea and vomiting. Some cases, often caused by *Campylobacter jejuni*, can be severe and the mortality is 0.6 % deaths/year (Danish Veterinary and Food Administration (DVFA) 2006). The Danish Veterinary and Food Administration have estimated that 80 % of the cases of campylobacteriosis are food-borne, mostly caused by contaminated poultry. Other sources of infections are contaminated water, poor handling of food, poor cooking, and/or poor kitchen hygiene (Corry *et al.* 2007, Luber *et al.* 2006, Wieland *et al.* 2006). Due to good growth adaptation an amount of 500 *Campylobacter* is sufficient to generate illness (Bashor *et al*. 2004).

Campylobacteriosis is a global disease and one of the most commonly reported food-born diseases in industrial countries (Johnsson *et al*. 2006, Hilmarsson *et al.* 2006). An increase in incidents has been observed in Denmark with 1100 cases in 1992 and 3700 cases in 2004 (MFCA 2005). In Denmark, Statens Serum Institute (SSI) has estimated that the real number of human cases is up to 20 times the reported number of cases. The most prevalent carrier of *Campylobacter* is fresh poultry (Hansson *et al.*2005, Zhao *et al.* 2006).

Due to the large economic and health consequences of campylobacteriosis, intervention programmes to reduce the disease rates have been initiated by Denmark (2003) and other developed countries. The programs involve several steps, especially for chicken: A) Monitoring and reducing the presence of *Campylobacter* during the feed-to-food process (Wieland *et al.* 2006), e.g. in water and feed at farms and during slaughtering (Sandberg *et al*. 2005), B) refrigerating meat quickly after slaughtering of the animals, and C) using chickens from *Campylobacter* negative broiler flocks for fresh meat products and meat from positive broiler flocks for frozen products (MFCA 2005). The programme has created an opportunity for Denmark, in the nearest future, to apply for special status in EU with regards to *Campylobacter*-free meat (DVFA 2006) and thereby increase the competitiveness of Danish products (Korsgaard *et al.* 2005). However, to obtain such a status, steps needs to be carried out in order to further reduce the presence of *Campylobacter*. Methods like bird washers with chlorinated water (James *et al.* 2000, Escudero-Gilet *et al.* 2005, Siragusa *et al.* 1995, Bashor *et al.* 2004) are used in some countries for decontamination of poultry, although not as effective as desired.

The SonoSteam® treatment is, however, an excellent candidate to improve the reduction of *Campylobacter*, due to the combination of steam and ultrasound, which are both generated in nozzles by supply of steam at high pressure. The ultrasound disturb the natural occurring zone of air closest to the surface, the laminar boundary layer, which restricts vapour and heat exchange across the surface. The ultrasound sets the air of the laminar zone in a state with intensified molecular oscillations, causing a destruction of the laminar boundary layer. When the laminar layer is destructed, hot steam can enter microstructures and pits in the surface and secure a fast heat transfer. The micro-organisms are quickly heated up and killed, so that the treatment can end before the heat affects the surface of the product.

The objective of this study was to determine the reduction of *Campylobacter* on freshly slaughtered chicken carcasses by treatment with a combination of steam and ultrasound (SonoSteam®).

2. Materials & Methods

2.1. Preparation of samples

A total of 60 freshly slaughtered chickens from *Campylobacter* positive flocks were included, 30 carcasses were non-treated references and 30 carcasses were treated with a combination of ultrasound at a level of 160 dB and frequency of 20-25 kHz (SonoSteam®) and steam at maximum output of 100 kg/h and temperature of 100° C. The SonoSteam® system using Stem-jet nozzles was a specifically developed pilot plant, installed temporarily in a container beside the slaughterhouse, in order to treat freshly slaughtered chicken carcasses and thus simulate an implementation in production line. The chickens were treated inside for 5 seconds, and on the outside, the chickens were treated from one side while turning around for 10 seconds. Treatments were carried out on carcasses taken from the production line prior to the inside-outside bird washer. After treatment, the carcasses were placed individually in sterile Stomacher® bags with filter (3500 ml) and preserved at 4° C ON until analysis. Two series of trials were performed.

2.2. Microbial analyses

The microbial analyses were performed by the Danish Institute for Food and Veterinary Research (DFVF) according to the NMKL-method (April 2005). In brief, carcasses were rinsed by adding 200ml of 0.1 % sterile buffered peptone salt (pH 7.2 ± 0.2 at 25°C; autoclaving at 121° C for 15 min.) and scrubbing for 2-3 min. Rinses were collected separately, transferred to a 250 ml centrifuge tube and centrifuged for 15 min. at 13,000 x g. The pellet was resuspended in 10 ml 0.1 % PBS and serial dilutions (10^{-1} – 10^{-4}) in 0.1 % PBS were prepared for determination of *Campylobacter* numbers. 100 µl from all the suspensions were transferred onto an Abeyta-Hunt-Bark agar plate with 0.01 % Triphenyl-tetrazoliumclorid. The inoculated plates were incubated at 41.5° C for 48 hours, *Campylobacter* colonies were counted, and the concentration given as CFU/ml.

3. Results and discussion

The effect on the level of *Campylobacter* on the surface of chicken carcasses by treatment with a combination of steam and ultrasound (SonoSteam®) was investigated in this experiment. The calculated concentrations of *Campylobacter* before and after treatment with SonoSteam® are outlined in table 1. The two trials showed a significant decrease in the level of *Campylobacter* from 3.60 and 4.43 $\log_{10}$ units (CFU/ml) to 1.67 and 1.34 $\log_{10}$ units (CFU/ml), respectively.

Table 1 Concentration of *Campylobacter* on freshly slaughtered chicken carcasses before and after one second of steam and ultrasound treatment (SonoSteam®).

n(group)=30	Reference (log_{10} CFU/ml)	SonoSteam® (log_{10} CFU/ml)	Reduction (log_{10} CFU/ml)	Significance
Trial 1	3.60 ± 0.67	1.67 ± 0.73	1.93	**
Trial 2	4.43 ± 0.51	1.34 ± 0.50	3.09	***
Mean	4.02	1.51	2.51	N.D.

The mean reduction of *Campylobacter* after SonoSteam® treatment was 2.51 log_{10} units, which is illustrated in figure 1. No visual organoleptic changes due to the treatment were observed.

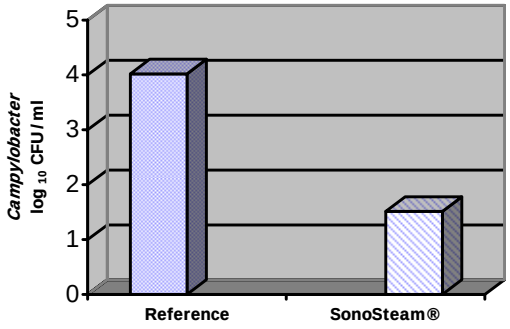

Figure 1 Concentration of *Campylobacter* on chicken carcasses treated with a combination of steam and ultrasound (SonoSteam®) for maximum 10 sec. as well as untreated references.

It has been estimated that a log 2 reduction in the *Campylobacter* concentration on poultry will lead to a 30-fold decrease in human cases of campylobacteriosis (Corry *et al.* 2007).

Bird washers are presently used for cleaning of chicken carcases. Bashor *et al.* (2004) tested traditional carcass washer systems without and with addition of the chemicals TSP and ASC. These systems reduced the *Campylobacter* concentration on carcasses log 0.5, 1.03, and log 1.26 CFU/ml, respectively. The combination of chlorinated washer and an anti-microbial treatment resulted in a reduction of *Campylobacter* by log 1.53 CFU/ml. The reductions obtained in this study indicate that SonoSteam® treatment would be more efficient against *Campylobacter* on the surface of raw meat. Additionally, the industry has excluded chemicals for decontamination of raw poultry. The focus has, therefore, been shifted to heat-based processes (James *et al*. 2000) such as SonoSteam®, which are applicable techniques for the food industry due to no use of chemicals or disposal waste products (Avens *et al*. 2002, Nutsch *et al.* 1997).

A significant reduction of *Campylobacter* on poultry, as obtained in this study, has also been achieved by treatment with hot water or steam. Purnell *et al.* (2004) (Escudero-Gilete *et al.* 2005) showed a significant reduction in *Campylobacter* on chickens by treatment with hot water (70°C) for 40 seconds. Another study indicates that when hot water is applied to poultry carcasses, total aerobic bacterial counts may be reduced by app. log 1-3 CFU/cm^2 (Sofos *et al.* 1998). However, the level of

aerobic micro-organisms should not be used as an index for *Campylobacter* due to a greater variance (Cason *et al.* 1997).

One problem with the use of hot water or steam is that the product easily is cooked by the treatment. It is vital that the treatment does not decrease the quality of the meat by changes in the surface structure (Avens *et al.* 2002, Smulders *et al.* 1998), and this has been achieved in the current study. Berrang *et al*. (2000b) could not achieve a reduction of *Campylobacter* on chicken carcasses by scalding without observing organoleptic changes. Due to such a change, the consumers will prefer raw meat that has been treated more gently, such as by the SonoSteam® technique. Hot water immersion and flowing steam methods are used for treatment of carcasses that are further processed into skinless poultry meat products where surface cooking is tolerable (Avens *et al.* 2002).

Another problem with currently known methods is the long treatment time, which also affects the surface changes. Morgan *et al.* (1996) achieved a log 2-4 reduction of *Listeria* on inoculated meat be treatment with steam pasteurisation (high pressure-temp 145°C, and vacuum) for less than one second. This method has been confirmed by Nutsch *et al*. (1997) and resembles the efficiency of SonoSteam®, obtained in this study. At both systems, the meat surfaces are briefly exposed to steam, but the steam-vacuum systems use temperatures below boiling (82.2° C) (Avens *et al.* 2002), in contrast to SonoSteam®.

Previous studies have indicated that reduction of *Campylobacter* on the surface of chickens by SonoSteam® treatment depends on the temperature of the skin and the initial level of *Campylobacter* contamination of the samples (data not shown). Treatment of cold chicken cut-outs resulted in lower reductions than the reductions obtained in this experiment with freshly slaughtered, warm chickens. It is possibly due to the poor growth conditions for *Campylobacter* at 4° C (Sandberg *et al.* 2005, Zhao *et al.* 2006) which results in a lower start concentration than in the case of freshly slaughtered, warm chickens. Further experiments should aim to be carried out with freshly slaughtered, warm chickens as in this study. Future studies should be performed aiming to develop the SonoSteam® technology, concerning optimisation of the nozzles as well as determination of optimal treatment times for different products.

4. Conclusion

Treatment of freshly slaughtered chicken carcasses with SonoSteam® for maximum 10 sec. efficiently reduced *Campylobacter* numbers on the surface with 2.51 $\log_{10}$ units. An in-line SonoSteam® prototype for implementation in a poultry slaughterhouse should be tested.

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