The influence of pig carcass processing of the efficacy of sponge swab sampling

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Abstract

The efficacy of different methods of sampling have been widely compared in the literature. Whilst it is recognised that swabbing and sponging leave a residual bacterial population, the levels that are left are difficult to evaluate and may be influenced by other factors such as changes to the skin due to processing. In this Food Standards Agency funded study we have used bacterial bioluminescence as a visual marker of the presence of bacteria to evaluate the efficacy of different sampling methods on the removal of bacteria. Pig skin was spiked with a strain of E. coli or Salmonella Typhimurium made bioluminescent by the introduction of the luxCDABE genes from Photorhabdus luminescens on a plasmid construct. Samples were visualized under a light sensitive camera before and after sponging or swabbing and the levels of the bacteria removed evaluated. Methods compared were agitated sponging, using cellulose acetate sponges, against traditional sponging and a double-swabbing technique, using cotton tipped bud swabs. Results indicate that damage to skin can lead to 'hot spots' of contamination, where residual bacteria are not easily removed by further physical abrasion.

Introduction

Microbiological sampling and testing of carcasses has been introduced in many countries to verify that HACCP schemes effectively control plant processing. Whilst many studies have compared the efficiency of different sampling methods (excision, sponging, wet-dry) (Hutchison et al., 2005; Pepperell et al., 2005), few studies have been undertaken on the efficiency of alternative sponge-sampling methods. The UK Food Standards Agency (FSA) requires that Salmonella sampling of carcasses can only be undertaken using sponges. These are seen as easier to use, particularly on a moving line, less affected by operator variability and as cost effective because only one set of sampling consumables is required for all of the statutory tests. For testing using sponges the recommended approach is to agitate the sponge by moving it by a few centimetres using a side-to-side movement (Anon, 2006). Here we evaluate the efficacy of agitated sponging against a technique in which multiple sponge passes are made through a delineated area and against wet-dry swabbing with cotton-tipped swabs.

To allow the removal of bacteria to be monitored easily, we have spiked pork rind with Escherichia coli or Salmonella Typhimurium engineered to carry the lux genes making the bacteria bioluminescent. The presence of such bacteria on a surface can then be viewed using a light sensitive camera.

Materials and methods

Sampling

Sponge sampling was carried out using cellulose acetate sponges; swabbing was carried out using cotton tipped bud swabs. Agitated sponge sampling was performed on a section of pork rind over a 10 x 10 cm area in a single pass. The sponge was agitated from side-to-side across the whole area in the fashion recommended by the FSA. Traditional sponge sampling was performed by rubbing the sponge firmly across the rind surface with 10 strokes in each of the horizontal and vertical directions, with no side-to-side agitation. Wet-dry swab sampling was undertaken by
rubbing a swab moistened in maximum recovery diluent firmly across the rind surface with 10 strokes in each of the horizontal, vertical and both diagonal directions. Swabs were rolled between the thumb and index finger as they were rubbed across the rind surface. Immediately, after rubbing with the moistened swab, the procedure was repeated within the sample template with a dry swab.

**Efficacy of carcass surface sampling methods**

Samples of pork rind were inoculated with an *Escherichia coli* or *Salmonella Typhimurium* strain which constitutively express luxABCDE genes from *Photorhabdus luminescens* on a plasmid construct. Bacteria were inoculated to a final concentration of approximately $1 \times 10^5$ cfu ml$^{-1}$. Following inoculation the pork rind was incubated at 37°C for 1 hour so that the bacteria could adhere to the pork rind surface. Before and after sampling, photographs were taken of the skin and sponge/swab using a Night Owl CCD camera (EG & G Berthold, Bad Wildbad, Ger.). Two minute integration times were used.

**Results**

**Agitated Sponge Sampling**

![Figure 1](image.png)

*Figure 1. Bioluminescence image of a 100cm$^2$ section pork rind inoculated with $1 \times 10^5$ cfu cm$^{-2}$ bioluminescent *E. coli* (A) prior to sampling (B) following agitated sponging.*

From Figure 1 comparison of the sample before and after agitated sponge sampling demonstrates a significant reduction in light output. This demonstrates the method removes a large proportion of the bacteria present on the rind surface. Examination of the sponges confirmed that bacteria had been removed and were present on the sponge surface (data not shown). The residual light on the rind surface appears to be associated with micro-topological features created by an undetermined aspect of the slaughter process. After two further rounds of sponging (data not shown), the reduction in bacterial bioluminescence, relative to the intensity of light emission visualized after one round of agitated sponging, was minimal. This suggests the remaining bacteria are firmly adhered to the surface.
Traditional sponge sampling

Post-sampling, the level of bioluminescence emitted from bacteria on the rind surface (Figure 2B) is not noticeably different to that remaining following agitated sponging (Figure 1B). However, the traditional sponging technique is more time consuming and involves more actions than agitated sponging making it a less easy to use method when sampling from carcasses in slaughterhouses during processing.

Wet-dry swab sampling

Following swab sampling there was very little change in the levels of bioluminescence produced, indicating that a substantial proportion of the inoculant remained on the rind surface. Bacterial removal by the swabs was confirmed by further testing.

Discussion

Although an essentially qualitative approach, the use of bioluminescent bacteria does give a simple evaluation of the effectiveness of different sampling methods. Both sponge sampling methods tested were more effective at removing bacteria than swab sampling, probably because of the larger surface area sponges have in contact with the skin. The agitated sponge method is also quicker, needs less skill to carry out and gives less variation between operators. Repeated sampling of an area never completely removed the bacteria, particularly where these were associated with micro-topological features. This suggests that some contamination may be firmly attached which may be protected from physical methods of removal such as washing.
Reference

