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Short communication

Development and testing of a prototype automatic trim sampler

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A B S T R A C T

The N60 method currently mandated by regulatory bodies in North America for routine testing of beef trim for *Escherichia coli* O157:H7 requires that 60 slices of beef, up to 375 g, be removed from combo bins containing up to 10,000 lb of beef trim. The objective of this study was to design and test a prototype automatic trim sampler that would reduce the resource demands of, and result in more representative sampling than, N60. Ten commercial combo bins were each sampled at five locations of each of four levels, by swabbing an area of 1000 cm^2 and by excision of up to five meat pieces with a total area of 100 cm^2. The samples were enriched in modified Tryptone Soy Broth supplemented with novobiocin at 20 mg/L, and tested for generic *E. coli* using real-time PCR. A prototype trim sampler was constructed and tested for recovery of aerobes, coliforms and *E. coli* and was compared to manual swabbing, using beef trim artificially contaminated with *E. coli*. Overall, 21.5% of excision samples and 38.0% of swab samples from combo bins were positive for *E. coli*. Of the 40 levels that were sampled, 50.0% were positive by excision, 70.0% were positive by swabbing. The sampler, designed based on swab sampling, could process trim of $\leq$1 kg. Of the 12 pairs of manual sampling and automatic sampling compared, $\geq$ 8 were not significantly different for recovering each of the three groups of indicator organisms ($P > 0.05$). The findings of this study show that swab sampling can be more sensitive and representative than excision sampling for recovering small numbers of *E. coli* from beef trim and the prototype sampler can have efficacy comparable to manual swabbing for recovering all three groups of indicator organisms from beef.
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1. Introduction

Verotoxigenic *Escherichia coli* (VTEC) were first recognized as pathogens of great public concern in 1982, following outbreaks associated with the serotype O157:H7 in the US (Riley et al., 1983). Cattle are natural reservoirs of VTEC, but they are asymptomatic carriers (Gill & Gill, 2010). It is then not surprising that *E. coli* O157:H7 was detected in up to 94% of samples from hides of cattle after hide opening but before hide removal in a commercial beef packing plant in the US (Arthur et al., 2004). Most bacteria on carcasses are deposited during the skinning operations where the transfer of bacteria from the hide to the previously bacteria free meat is inevitable (Bacon et al., 2000; Bell, 1997; Nottingham, 1982, pp. 13–65). Thus, skinned carcasses can be contaminated with *E. coli* O157:H7. Despite the implementation of effective antimicrobial interventions at beef plants, complete elimination of *E. coli* O157:H7 from beef is not yet attainable.

Due to the severity of illness caused by *E. coli* O157:H7 and its association with ground beef, the US Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) declared the organism a food adulterant in 1994 (Taylor, 1994). Shortly after the declaration, FSIS initiated a program to test for its presence in ground beef and subsequently in 2007, and expanded the testing to include beef trim intended for use in raw ground beef or beef patty products at the slaughter establishments that produced that trim (USDA-FSIS, 2013). Beef trim (trimmings) consists of small portions of beef that are trimmed away during the fabrication of primal and subprimal cuts from large portions of carcass sides (Arthur, Bono, & Kalchayanand, 2014). They are sorted, often according to visual observation of the fat content, and are collected into combo bins, which, when filled, can weigh up to 2000 lb each. The current sample collection method mandated by FSIS and the Canadian Food Inspection Agency (CFIA) for testing of beef for *E. coli* O157 is called

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N60 in which 60 slices of meat (6.25 g/slice) from a production lot are obtained by manual excision (CFIA, 2015; USDA/FSIS, 2014). A production lot contains up to 5 combo bins. At present the process at large beef packing plants in Canada requires 6–8 individuals per shift directly involved with sampling and another 2 individuals per shift involved with the paperwork, lot tracking, delivery of samples to the lab, etc. At current wage rates in Canada the cost of sampling exceeds $1 million per year when both shifts at large facilities are considered. Thus, the N60 method is very resource intensive. In addition, it is difficult to obtain a truly representative sample by the N60 method for a number of reasons. Notable among these are the difficulty inherent to accessing meat for sampling anywhere other than in the top of the bin, 75 g of meat being a very small proportion of the 2000 lb in a bin, and inhomogeneous distribution of the pathogen in the bin. In addition, despite the severity of illness caused by *E. coli* O157:H7, it is found on beef at very low prevalence, and at very low numbers when it is present. The largest beef recall in Canadian history was triggered by detection of *E. coli* O157:H7 by the USDA in trim that had tested negative for the organism by routine testing at the plant where the trim was produced (Lewis, Corriveau & Usborne, 2013). The recall led to the closure of the beef plant which represented 35% of Canadian beef processing capacity. Therefore, automated approaches with improved representation of samples could save resources and reduce the amount of contaminated meat that would otherwise enter the market.

For automated sampling equipment to be devised, knowledge of whether sampling by swabbing can be used instead of technically more difficult excision sampling is required. The efficiency of swab sampling can be comparable with that of excision for recovering bacteria from carcasses of large animals (Gallina et al., 2015; Gill & Jones, 2000; Gill, Badoni, & McGinnis, 2001). Information on using swab sampling to recover small numbers of bacteria from manufactured beef, however, is largely lacking. Thus, this study was staged in two phases: 1. to determine whether automated sampling equipment would require excision sampling, in order to test beef trim at the level of contamination routinely attained at Canadian beef processing facilities; and 2. to construct and test the automated sampling equipment developed based on the findings from phase 1.

2. Materials and methods

2.1. Recovery of generic E. coli from commercial trim by swab and excision sampling

2.1.1. Sample collection

Ten combo bins of beef trim were obtained from a Canadian beef packing plant which processes feedlot cattle at rates up to 280 head per hour, through a process described in previous studies (Yang, Badoni, Tran, & Gill, 2015; Yang, Badoni, Youssef, & Gill, 2012). Each combo bin contained approximately 2000 lb of trim with a fat to lean ratio of 50:50. The combo bins were collected from the plant on 6 days at approximately weekly intervals, with one bin during each of the first two weeks and two bins during each of the following 4 weeks being collected. Upon collection from the plant, the combo bin(s) was transported in a refrigerated truck to a laboratory for further processing. The combo bins were designated as B1-10 in the order they were sampled.

Samples were collected from the four corners as well as the centre of the uppermost surface of each bin, by both excision and swabbing (Fig. 1). For excision, five thin strips measuring approximately 10 cm × 2 cm each were excised from trim in each of the five areas. The total weight of meat obtained from each area was ≤50 g. An area of approximately 1000 cm^2 of trim in each area from which strips of meat were excised was also swabbed using a sponge

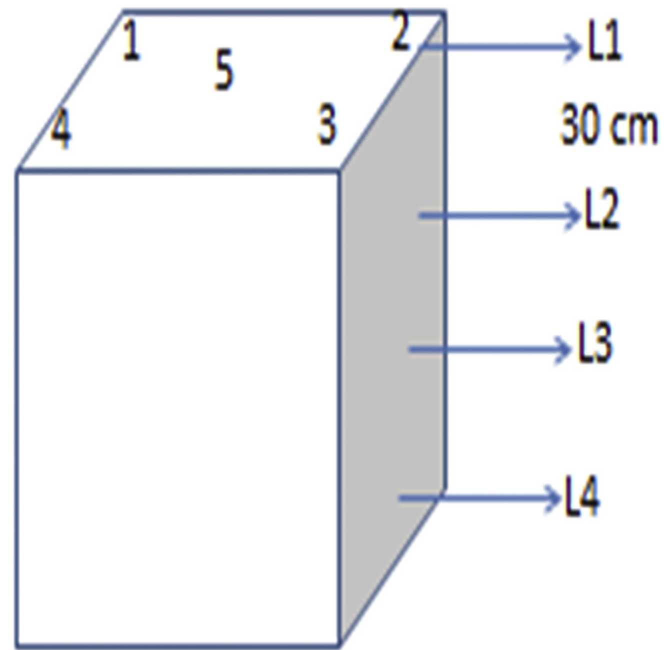

Fig. 1. Schematic diagram of sites of sample collection from combo bins. Samples were collected from four corners (1–4) and the centre of each of four levels (L1-4) of each combo bin of trim.

(Speci-sponge; VWR Canlab, Edmonton, Alberta, Canada) premoistened with 7 ml of 0.1% peptone water (Difco, Becton Dickinson, Sparks, MD). The meat sample or swab from each sampling site was placed in a sterile stomacher bag (Gill et al., 2001). Trim was removed from the bin until a fresh surface approximately 30 cm below the previous top surface was exposed. The bin edges were cut to make the new surface readily accessible. Five samples from the newly exposed surface were collected by excision or swabbing as before. The removal of meat and collection of samples were repeated for surfaces approximately 60 cm and 90 cm below the previous top surface. All stomacher bags containing samples were placed on ice and were transported to the laboratory for further analysis. A total of 200 samples were collected from excision or swabbing.

2.1.2. Quantification of E. coli in enrichment cultures

Ten ml of 0.1% peptone water was added to each swab sample which was then pummeled in a stomacher for 2 min. Five ml of the swab fluid, approximately half of the total volume, was withdrawn from the stomacher bag and was filtered through a hydrophobic membrane grid filter (HGMF; Neogen, Lansing, MI). The filter was placed on a Lactose Monensin Glucuronate (LMG; Neogen) and incubated at 35 °C for 24 h. Subsequently, the filter was transferred to a plate of buffered MUG (BMA; Neogen). After incubation at 35 °C for 3 h, the filter was examined under long-wavelength UV light and the squares containing blue-white fluorescent colonies were counted as *E. coli* (Liu, Youssef, & Yang, 2016). This method is referred as direct filtration in this study.

One hundred or 250 ml of modified Tryptone Soy Broth (mTSB; Oxoid, Nepean, Ontario, Canada) supplemented with 20 mg/L novobiocin (N; Oxoid) was added to each stomacher bag containing a pummeled swab or meat pieces, respectively. The stomacher bags were placed into a plastic bag rack and incubated at 42 °C for 18–24 h, to enrich for *E. coli* (Yang, Badoni, Wang, & Gill, 2014). A 1.5 ml portion of each enrichment culture was withdrawn and centrifuged at 1500×g for 1 min to pellet large debris. One ml

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