Use of Steam Condensing at Subatmospheric Pressures To Reduce *Escherichia coli* O157:H7 Numbers on Bovine Hide

J. M. McEvoy,1* A. M. Doherty,1 J. J. Sheridan,1 I. S. Blair,2 and D. A. McDowell2

1Teagasc, The National Food Centre, Castleknock, Dublin 15, Ireland; and 2University of Ulster, Jordanstown, Northern Ireland

**ABSTRACT**

This study used a laboratory-scale apparatus to apply subatmospheric steam to bovine hide pieces inoculated with *Escherichia coli* O157:H7 in maximum recovery diluent (MRD) and in high-liquid content and low-liquid content fecal suspensions (HLC fecal and LLC fecal, respectively). The survival of the organism in fecal clods, which were stored for 24 days in a desiccated state, was assessed. Inoculated fecal clods were also treated with subatmospheric steam. Steam treatment at 80 ± 2°C for 20 s reduced *E. coli* O157:H7 concentrations on hide inoculated to initial concentrations of approximately 7 log10 CFU/g by 5.46 (MRD inoculum), 4.17 (HLC fecal inoculum), and 5.99 (LLC fecal inoculum) log10 CFU/g. The reductions achieved in samples inoculated with LLC feces were larger than in samples inoculated with HLC feces (*P < 0.05*). Treatment at 80 ± 2°C for 10 s resulted in significantly smaller reductions (*P < 0.05*) on hide pieces of 2.54 (MRD), 1.94 (HLC fecal), and 2.15 (LLC fecal) log10 CFU/g. There were no significant differences among the reductions observed in all inoculum types in samples treated for 10 s. *E. coli* O157:H7 inoculated in fecal clods to 7.78 log10 CFU/g and stored at 4 or 15°C survived for at least 24 days. Steam treatment (20 s) of 3-day-old clods reduced surviving *E. coli* O157:H7 numbers from 4.20 log10 CFU/g to below the limit of detection of the assay used (1.20 log10 CFU/g). This study shows that steam condensing at or below 80 ± 2°C can reduce *E. coli* O157:H7 when present on bovine hide, reducing the risk of cross contamination to the carcass during slaughter and dressing.

Control of *Escherichia coli* O157:H7 during beef processing is essential to minimize the health risk the organism poses to consumers. Studies have described procedures to reduce the incidence and numbers of pathogens on beef carcasses (7, 9, 10, 14, 19, 20, 23); however, no commercially acceptable methods currently available can guarantee the complete elimination of *E. coli* O157:H7 from carcasses. In the absence of such guarantees, strategies to protect consumers must focus on preventing initial transfer of the organism onto carcasses.

The hide is widely recognized as a significant source of contamination that can transfer to the carcass during dressing operations (4, 11, 12). Adherence to hygienic practices by operatives responsible for removing the hide can reduce the extent of cross contamination (16, 18). However, despite good practices, cross contamination remains inevitable (4, 12).

Methods to reduce pathogen numbers on the hide, and therefore reduce the reservoir available for transfer to the carcass, have been investigated. On a laboratory scale, a chemical dehairing treatment significantly reduced *E. coli* O157:H7 and *Salmonella* Typhimurium numbers on the hide (6). In another study, washing removed feces inoculated with *E. coli* O157:H7 from a delimited area of the hide (5). However, the success of these methods, which relies to a greater or lesser extent on the physical removal of contamination, could be affected by the accumulation of dirt on the hide. Depending on season and husbandry practices, the hide can be contaminated with moderate or heavy coatings of dirt and feces, which can cover almost its entire surface (1, 2, 25). In some cases, the feces can accumulate and harden to form fecal clods (1, 2).

Because the physical removal of accumulated contamination can be difficult to achieve, the inactivation of pathogens such as *E. coli* O157:H7 in situ might be required to achieve decontamination. Steam methods have advantages for in situ decontamination because it is an effective heat transfer medium and it gives out large quantities of energy when it condenses (17).

A test system using steam condensing at subatmospheric pressure to decontaminate heat-sensitive products has been described (3, 13). Using this system, condensing vapor rapidly raises the surface temperature of the product, and posttreatment rapid cooling is achieved by removing condensed liquid under vacuum. The present study used the subatmospheric pressure steam decontamination test system, with steam condensing at 80°C for intervals of 10 and 20 s, to treat bovine hide pieces inoculated with *E. coli* O157:H7 in fecal and nonfecal suspensions. The study also examined the persistence of *E. coli* O157:H7 in dried fecal clods and the survival of this organism in fecal clods during treatment with steam condensing at 80°C for 20 s.

**MATERIALS AND METHODS**

**Steam treatment equipment.** The steam treatment equipment, as designed at the University of Bristol (UK), Food Refrigeration and Process Engineering Research Centre, consisted of a...
process vessel (glass bell jar and stainless steel base plate) in an implosion guard (Fig. 1). The chamber could be depressurized by connection to a vacuum pump through a solenoid valve located on the base plate. This process provided conditions under which steam, introduced through a steam inlet valve, condensed at temperatures below 100°C. Control of the vacuum solenoid valve during treatment enabled the temperature within the chamber to be maintained at preselected values ±2°C. Evaporative cooling in the chamber could be achieved under a vacuum of 1.2 to 2.0 kPa.

Preheating the bell jar and the base plate. The bell jar and base plate were preheated before insertion of hide samples to minimize heat loss during treatment as a result of latent heat transfer. Because of the difficulty of attaching probes to the bell jar, only the base plate temperature was measured. It was continuously monitored using a data logger (Squirrelwise, Grant, UK) connected to T-type thermocouples fixed to its surface. The steam inlet valve was opened to allow steam into the process vessel, raising the base plate temperature to 100°C for 1 min. The steam supply valve was then closed, and the process vessel was evacuated to a pressure of 1.2 to 2.0 kPa by opening the vacuum supply solenoid. The vacuum pump was then switched off, and the chamber was flooded with air from the external atmosphere. Sample treatment was carried out within 3 min of the completion of preheating.

Treatment. The sample to be treated was suspended from a support bar in the process chamber. The condensed vapor temperature in the chamber was monitored using three T-type thermocouples attached to the surface of a polytetrafluoroethylene block placed beside the hide sample. Vacuum was applied to reduce the internal pressure in the chamber to 1.2 to 2.0 kPa. Steam was introduced to raise the temperature to 80 ± 2°C. Treatment intervals (10 or 20 s) were measured from the point at which the temperature of the block reached 80 ± 2°C. During treatment, the temperature was maintained at 80 ± 2°C. After each treatment interval, the vessel was cooled by evacuation as previously described. Samples were removed aseptically and examined as described below.

Organism. A mutant nontoxigenic strain of *E. coli* O157:H7 (NCTC 12900) resistant to nalidixic acid (Sigma Chemical Co., St. Louis, Mo.) (50 μg/ml) and streptomycin sulfate (Sigma) (1,000 μg/ml) was prepared (21). The organism was maintained on cryoprotectant beads (Technical Service Consultants Ltd., Heywood, Lancashire, UK) at −34°C. A culture of the organism was produced by aseptically transferring one bead containing *E. coli* O157:H7 into 9 ml of tryptic soy broth (Oxoid Ltd., Basingstoke, Hampshire, UK) and incubating at 37°C for 20 to 24 h. From this culture, 0.1 ml was transferred to 100 ml of fresh tryptic soy broth and incubated at 37°C for 16 h. The resultant cells were recovered by centrifugation at 3,000 × g (Eppendorf Centrifuge 5403, Hamburg, Germany), washed three times in maximum recovery diluent (MRD), and resuspended in 100 ml of MRD. This process yielded an inoculum containing approximately 8.5 log_{10} CFU/ml.

Collection and testing of bovine feces. Bovine feces were collected immediately after defecation from cattle at The National Food Centre. Samples of collected feces were examined for the presence of *E. coli* O157:H7 resistant to streptomycin sulfate and nalidixic acid. This was achieved by duplicate plating of 1 ml of a 1:10 suspension of feces onto sorbitol MacConkey agar supplemented with 50 μg/ml nalidixic acid and 1,000 μg/ml streptomycin sulfate (SMAC), incubation at 37°C for 20 to 24 h, and examination for the presence of sorbitol nonfermenting colonies.

Use of steam to reduce *E. coli* O157:H7 counts on bovine hide. A section of approximately 1 m², including the area on either side of the backbone, was taken from a bovine hide immediately after dehiding and was transported to the laboratory where it was stored at 2°C for up to 24 h. From this section of hide, visibly clean pieces measuring 9 by 10 cm were removed and inoculated. An MRD inoculum was prepared as follows. A 5-ml aliquot of the *E. coli* O157:H7 culture, suspended in MRD, was spread onto the surface of two 9- by 10-cm hide pieces using a sterile spatula to give a count of ca. 7 log_{10} CFU/g. Inoculated hide pieces were allowed to stand at room temperature for 15 min.

A high-liquid content fecal inoculum (HLC fecal) was prepared as follows. A 50-ml aliquot of the *E. coli* O157:H7 culture suspended in MRD was added to 16.5 g of fresh bovine feces and mixed thoroughly by hand. This resulted in feces with a high liquid content. A 5-ml aliquot of this inoculum was spread onto the surface of two 9- by 10-cm hide pieces using a sterile spatula to give a count of ca. 7 log_{10} CFU/g. Inoculated hide pieces were allowed to stand at room temperature for 15 min.

A low-liquid content fecal inoculum (LLC fecal) was pre-
pared as follows. A 5-ml aliquot of the *E. coli* O157:H7 culture suspended in MRD was added to 50 g of fresh bovine feces and mixed thoroughly by hand. This resulted in feces with a low liquid content. A 5-g aliquot of this inoculum was spread onto the surface of two 9- by 10-cm hide pieces using a sterile spatula to give a count of ca. 7 log_{10} CFU/g. Inoculated hide pieces were allowed to stand at room temperature for 15 min.

For each inoculum type (MRD, HLC fecal, LLC fecal), one hide piece of each pair was examined without steam treatment. This (untreated) sample was placed in a sterile stomacher bag, diluted 1:5 (wt/vol) in MRD, pummelled in a stomacher (Colworth Stomacher 400, Colworth, London, UK) for 60 s, plated in duplicate 0.1-ml aliquots on streptomycin sulfate and incubated at 37°C for 24 h. Sorbitol nonfermenting colonies were enumerated as *E. coli* O157:H7.

For each inoculum type, the second hide piece of a pair was steam treated at intervals of 10 or 20 s, aseptically removed from the treatment vessel, and analyzed as described above. Reductions were calculated by subtracting the counts on steam-treated hide samples from those on untreated hide samples.

**Survival of *E. coli* O157:H7 in dried fecal clods.** The *E. coli* O157:H7 culture suspended in MRD was mixed thoroughly with fresh bovine feces by hand in a ratio of 1:10 (vol/wt) to form inoculated feces with an initial log_{10} CFU/g. Inoculated hide pieces were allowed to stand at room temperature for 15 min. For each inoculum type, the second hide piece of a pair was steam treated at intervals of 10 or 20 s, aseptically removed from the treatment vessel, and analyzed as described above. Reductions were calculated by subtracting the counts on steam-treated hide samples from those on untreated hide samples.

**Use of steam to reduce *E. coli* O157:H7 numbers in dried fecal clods.** A fecal clod inoculum, prepared as previously described, was dispensed in 20-g amounts, in triplicate, to include one end of a 10-cm piece of string (Fig. 2) and was placed in a fan-assisted incubator at 15°C for 3 days. After this period, fecal clods had a maximum thickness of 0.5 cm and were sufficiently solid to be suspended by the integral string during subsequent processing.

After 3 days, one of the triplicate fecal clods was placed in a sterile container and the string was aseptically removed. The clod was diluted 20-fold (wt/vol) in MRD using a gravimetric diluter (Watson Marlow Ltd., Falmouth, Cornwall, UK) and mixed to obtain a consistent suspension. This was treated as the control. The remaining two clods were each treated with steam for 20 s as described above. Steam-treated fecal clods were sampled in a similar manner as the control fecal clods above. *E. coli* O157:H7 numbers were estimated as described above. Reductions were calculated by subtracting the counts on steam-treated clods from those on untreated clods.

**Statistical analysis.** Three replicates in duplicate were conducted on paired hide pieces from each inoculum type (MRD, HLC fecal, and LLC fecal) at each treatment interval (10 and 20 s). The studies examining the survival of *E. coli* O157:H7 in fecal clods and the use of steam to reduce *E. coli* O157:H7 numbers in fecal clods were replicated three times. Statistical analyses were performed using Genstat Version 5 Release 3 (22). Nonlinear regression was used to fit the exponential models of *E. coli* O157:H7 decay. Analysis of variance was carried out using a linear model where the dependant variable was the reduction in log_{10} CFU/g following steam treatment. The independent variables were time, inoculum type, medium, and their interaction.

**RESULTS AND DISCUSSION**

**Use of steam to reduce *E. coli* O157:H7 counts on bovine hide.** Process parameters and their effects on *E. coli* O157:H7 numbers on bovine hide are presented in Table 1. The 20-s time treatment reduced *E. coli* O157:H7 on the hide by 4.17 to 5.99 log_{10} CFU/g. A similar magnitude of reduction was reported when using a chemical dehairing treatment to decontaminate excised sections of bovine hide (6). Those authors used 10% sodium sulfide to reduce *E. coli* O157:H7 from 5.3 log_{10} CFU/cm^2 to below the limit of detection (0.5 log_{10} CFU/cm^2).

The 10-s time treatment reduced *E. coli* O157:H7 numbers on the hide by 1.94 to 2.54 log_{10} CFU/g. The magnitude of reduction is similar to that achieved using a cold water spray treatment for 3 min, which reduced *E. coli* O157:H7 by ca. 2 log units (5). In that study, the physical

**TABLE 1. Process parameters and their effects on *E. coli* O157:**

<table>
<thead>
<tr>
<th>Treatment time</th>
<th>Heat-up time</th>
<th>Cool-down time</th>
<th>Overall duration</th>
<th>Inoculum type</th>
<th>Reductions (log_{10} CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
<td>10</td>
<td>30</td>
<td>MRD</td>
<td>2.54 (0.67)</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>9</td>
<td>29</td>
<td>HLC fecal</td>
<td>1.94 (0.67)</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>8</td>
<td>28</td>
<td>LLC fecal</td>
<td>2.15 (0.67)</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>13</td>
<td>43</td>
<td>MRD</td>
<td>5.46 (0.62)</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>13</td>
<td>43</td>
<td>HLC fecal</td>
<td>4.17 (0.62)</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>13</td>
<td>43</td>
<td>LLC fecal</td>
<td>5.99 (0.62)</td>
</tr>
</tbody>
</table>

a Treatment at 80 ± 2°C.
b Time to raise the temperature to 80 ± 2°C.
c Time to cool down the vessel from 80 ± 2°C to 60°C.
d The combined heat-up, treatment, and cool-down times (df 57).
e Standard error reported in parentheses.
TABLE 2. Mean weight (g) and \( a_w \) of fecal clods following storage for periods ranging from 1 to 24 days at 4 and 15°C

<table>
<thead>
<tr>
<th>Day</th>
<th>4°C</th>
<th>15°C</th>
<th>4°C</th>
<th>15°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.0</td>
<td>2.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>0.75</td>
<td>0.29</td>
<td>0.96</td>
<td>0.67</td>
</tr>
<tr>
<td>2</td>
<td>0.31</td>
<td>0.23</td>
<td>0.72</td>
<td>0.25</td>
</tr>
<tr>
<td>3</td>
<td>0.31</td>
<td>0.24</td>
<td>0.51</td>
<td>0.34</td>
</tr>
<tr>
<td>4</td>
<td>0.27</td>
<td>0.24</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>0.28</td>
<td>0.26</td>
<td>0.41</td>
<td>0.32</td>
</tr>
<tr>
<td>8</td>
<td>0.26</td>
<td>0.26</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>0.26</td>
<td>0.21</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>24</td>
<td>0.26</td>
<td>0.26</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

removal method that was employed may have relocated the microorganisms onto another part of the hide. This does not occur with steam treatment because the microorganisms are inactivated in situ.

Using the MRD inoculum, the reductions following the 20-s time treatment were significantly greater than those using the 10-s time treatment (\( P < 0.05 \)). This is also true of the HLC fecal inoculum (\( P < 0.05 \)) and the LLC fecal inoculum (\( P < 0.05 \)). This effect of treatment time could be due to the high-density inoculum used (7 log_{10} CFU/g). Population density might afford protection to bacteria (15); hence, although the treatment temperature is sufficient to inactivate the microorganism, a longer treatment time is required to reach and inactivate protected cells.

A significantly greater reduction in \( E. coli \) O157:H7 numbers was achieved when using the 20-s time treatment on hide with the LLC fecal inoculum than on the hide with the HLC fecal inoculum (\( P < 0.05 \)). This indicates that the nature of the suspending medium might influence survival rates. It was found that when treating carcasses with hot water and steam, the aerobic plate count was reduced to a lower level at uninoculated sites than at sites inoculated with feces (10), possibly because of a reduced rate of heat transfer through the inoculum suspension. During steaming, the rate of heat transfer from the site of vapor condensation to the target site will decrease as the resistance to heat transfer increases. Such resistances could be in the form of either liquid (MRD) or solid (fecal) matter, through which heat must pass to reach the target site. It is possible that the increased liquid content of the HLC fecal inoculum offered a greater resistance to heat transfer than the LLC fecal inoculum. The resistance of moisture to heat transfer is minimized during pasteurization of beef carcasses by removing excess moisture using blowers before steaming (19).

Survival of \( E. coli \) O157:H7 in dried fecal clods. The weights and \( a_w \) of fecal clods assessed at intervals during storage at 4 and 15°C are presented in Table 2.

The weight of fecal clods decreased rapidly within the first 3 days of storage, whereas between days 3 and 24, there was little change in their weight. The \( a_w \) values were reduced to 0.41 and 0.32, respectively, following 7 days of storage. Similar \( a_w \) values (0.39 to 0.46) were reported in feces that had been stored for >40 days (26). The present study also noted a decrease in the rate of decline of \( E. coli \) O157:H7 numbers in fecal clods stored at 4 and 15°C for up to 24 days (Figs. 3 and 4). Extrapolation of the exponential decay curves, which are approaching their asymptote between days 3 and 24, would suggest that \( E. coli \) O157:H7 remains viable in fecal clods well in excess of 24 days. Hence, \( E. coli \) O157:H7 was reported as surviving for 49, 56, and 70 days in feces stored at 37, 22, and 5°C, respectively (26). The survival of \( E. coli \) O157:H7 following storage in a dried state at ca. 19°C for 20 days has also been reported (8). Although carried out under controlled conditions, the present study demonstrates the ability of the organism to survive in feces for prolonged periods of time, even in a desiccated state. Therefore, \( E. coli \) O157:H7 shed onto the hide in feces could remain viable for extended periods, even in the dried fecal clods that form on the hide over time.

Use of steam to reduce \( E. coli \) O157:H7 counts in dried fecal clods. The process parameters during the 20-s treatment of fecal clods were identical to those observed during the 20-s treatment of hide pieces.

The treatment reduced \( E. coli \) O157:H7 numbers in clods stored for 3 days at 15°C, from 4.20 log_{10} CFU/g to below the limit of detection of the assay used (1.20 log_{10} CFU/g). Reductions were achieved despite the possibility that the organism had an increased thermotolerance under
the low a_w conditions. It has been reported that because of the stability of proteins in a dry state, the absence of water increases the heat resistance of microorganisms (15).

Assuming that E. coli O157:H7 was present throughout the clod, and not just on the surface, this study demonstrated the ability of condensing steam to indirectly (by the process of conduction) reach target areas such as the center of a fecal clod, which might not be accessible to chemicals and could be difficult to remove physically.

The successful application of steam to decontaminate bovine hide on a commercial scale depends on a number of issues. Because the hide is an important by-product of beef production, the treatment should not affect the structure or composition of the hide and the quality of derived leather. Further studies will examine the effect of steam treatment on hide quality. Rapid heating, control at the desired temperature, and rapid cooling could be difficult to achieve on a commercial scale, and alternatives to vacuum control might be required. Rapid heating and cooling have been reported for the commercial-scale steam treatment of beef carcasses in an environment without pressure control (10, 14, 19, 20, 23). Nutsch et al. (20) reported an increase in the surface temperature of beef carcasses to 82.2°C within 1.5 to 4.5 s of commencing steam treatment. Following treatment, rapid cooling of the carcass surface to temperatures below 40°C in ca. 4 s was achieved using a cold water spray. Precise temperature control below 100°C for extended periods of time without vacuum control has also been reported during steam treatment. Woltersdorf and Mintzlaflf (27) described an apparatus used to scald pig carcasses using condensation. Using a mixture of steam and air, the temperature was maintained at 60 to 61.5°C for 420 s. The presence of air in steam lowers its condensation temperature (17), and by controlling the ratio of the two, condensation temperatures below 100°C can be achieved for extended periods.

Hide decontamination is a highly desirable critical control point during beef processing because it offers a genuine opportunity to prevent pathogens such as E. coli O157:H7 from contaminating edible carcass tissue. This study has demonstrated that steam, condensing at temperatures at or below 80 ± 2°C, can reduce E. coli O157:H7 numbers when present in fecal and nonfecal suspensions on bovine hide. Treatment time is significant, with a greater reduction at the 20-s than at the 10-s time interval. E. coli O157:H7 survives well in a desiccated state in feces; therefore, it can be expected in fecal clods on the hides of animals presented for slaughter. However, the 20-s time treatment described in this study can penetrate into the fecal clods, reducing the numbers of this pathogen by ≥3.0 log_{10} CFU/g.

ACKNOWLEDGMENTS

The authors thank Dr. Catherine Logue for her assistance during the preparation of this paper. We acknowledge with gratitude, grant aid under the Food Sub-Programme of the Operational Programme for Industrial Development. The program is administered by the Irish Department of Agriculture and Food and supported by Irish national and EU funds.

REFERENCES


