Verification of the Level of Microbiological Control for the Slaughter and Cooling Processes of Beef Carcass Production at a High-Line-Speed Abattoir

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ABSTRACT

Methods are described which were used to verify the microbiological adequacy of the processes of production and chilling of carcasses at a high-line-speed abattoir. Ten excision samples (5 by 5 by 0.2 cm) were taken from each of 16 to 20 carcasses for each evaluation of these processes. Twelve monthly evaluations were made for the slaughter of steers, heifers, and cows and additional evaluations for each of the slaughter of cows and the chill process of carcasses. The ranges of the estimated mean log10 most probable number of growth units per square centimeter (LMPN, for 236 carcasses) and Escherichia coli per square centimeter (LEC, for 240 carcasses) enumerated by hydrophobic-grid membrane filter technology for the 12 monthly evaluations of the slaughter floor were 1.11 to 1.62 (LMPN) for single samples and 0.20 to 0.65 (LEC) for pooled samples. Based on a published advisory scale for the slaughter floor the aerobic bacterial counts reflect a cleanliness level of “excellent” to “good.” For single evaluations of cow carcasses at the end of slaughter and of chilled carcasses the mean LMPN was 1.78 (“good”) and 1.40 respectively. From pooled samples of each of the 236 steer, heifer, and cow carcasses the pathogen E. coli O157:H7 was identified by polymerase chain reaction on one carcass whereas Listeria monocytogenes was identified on 14 carcasses. Verocytotoxigenic E. coli (6 isolates) and L. monocytogenes were not isolated from the same carcasses. These low isolation rates dictate a large sample size and therefore these pathogens are excluded from use to routinely verify the workings of hygienic processes. This control of cleanliness was measured directly by aerobic bacterial counts on groups of carcasses at the end of the slaughter floor line and after carcass cooling. In addition, the impact of the process of carcass production on hygiene was indirectly measured in this study by counting coliforms and Escherichia coli from pooled samples for groups of carcasses. Counts of both coliforms and E. coli are direct measures of fecal contamination and thus indirect measures of hygiene.

Our method of evaluating the cleanliness (measured by aerobic bacterial counts) of the slaughter process by hydrophobic-grid membrane filtration (HGMF) technology (HGMF, ISO-Grid, QA Life Sciences Inc., San Diego, CA) has been used in studies of groups of carcasses to evaluate heifers and steers, carcass washing, the slaughter process of six abattoirs in Alberta, and the cooling process of carcasses (15–18).

In this study, we describe twelve monthly applications of this method to evaluate the cleanliness of the slaughter process of carcass production (heifers, steers, and cows) up to the cooler at a high-line-speed abattoir in Alberta; the microbiological control of the processes of carcass production and cooling at another high-line-speed abattoir in Alberta has been published (18). HGMF aerobic bacterial
counts of the present studies were also used to measure the cleanliness of carcasses from similarly processed cows and of carcasses after the cooling process. The database of the above processes and previous slaughter process evaluations will be used to refine estimates of the final sample size required for process evaluations at abattoirs in Alberta. Samples collected after the slaughter process of heifers and steers were also used for the enumeration of coliforms and *Escherichia coli* and to determine the presence of verotoxigenic *E. coli* (VTEC) and *Listeria monocytogenes*. These pathogens might serve as a measure of the impact of control processes in beef slaughter on hygienic consequences, where hygiene is defined as the science of health and its preservation. This report includes the first studies of pathogen detection on beef carcasses and an evaluation of the slaughter process of cows in Canada. In a study at another high-line-speed abattoir in Alberta the same pathogens were identified on subprimal cuts before bagging and boxing (18).

**MATERIALS AND METHODS**

**The slaughter process**

At the time of this study the abattoir operated without a HACCP system, but an industry-developed quality-control program was in place. Surfaces of carcasses were trimmed on the slaughter floor (thirteen trimmers for the lateral surfaces of carcasses) and in the holding cooler (5 trimmers) to meet the zero-tolerance standard for fecal material. AAFC inspection followed the detailed Canadian document for carcass production from heifers and steers at abattoirs operating at high line speeds (2). The line speed for heifers and steers ranged from 200 to 290 carcasses per h.

**The carcass-cooling process**

After washing, carcasses passed through the spray water (chlorine 1 to 2 ppm) cooler before entering the holding cooler. Carcasses were sampled after the trim station in the holding cooler within 1 h of entering this cooler and 1 or 6 h before leaving the cooler for shipment by road transport. Two of 17 carcasses sampled were from cows.

**Group evaluation of carcasses at the end of the slaughter floor line**

Monthly evaluations were conducted from April 1994 to March 1995 as previously described (14, 15). Briefly, split carcasses were moved by plant staff at their discretion from the moving line to a secondary line for sampling at the end of the slaughter floor line after the carcass wash and just before entering the spray cooler. Of the 236 carcasses sampled for the 12 monthly evaluations, 7 (2.5%) had been on the "held" rail because of pathological defects, 21 (9%) had been evaluated for visual demerits as part of the quality-control program and 29 (12%) carcasses came from cows. The number of cows sampled was proportional to the number of cows, heifers, and steers slaughtered at this abattoir. This proportion was observed for each evaluation. The daily slaughter process called for cows to be slaughtered after cows, heifers and steers. Excision samples (5 by 5 by 0.2 cm) were taken from the leading side at 10 designated sites (see Table 1 and reference (14), Fig. 3) on each of 20 carcasses per evaluation (16 carcasses for the evaluation in February 1995). Aerobic mesophilic bacteria were enumerated automatically with an M101 HGMF Interpreter System (Richard Branker Research Ltd., Ottawa, Ontario) using hydrophobic-grid membrane filter (HGMF) technology. Estimates of the variability of the log$_{10}$ of the most probable numbers of growth units (MPNGU) per square centimeter at sites of carcasses from 6 abattoirs in Alberta indicated that 15 carcasses were needed to estimate the mean within the desired precision level of 0.5 log units with 95% probability (17). For each evaluation, samples were taken on three consecutive days from 4 to 8 carcasses per day. At the abattoir, HGMF were incubated (2-ml inoculum taken from 30 ml of 0.1% peptone—1% Tween 80 after 10 s of shaking the sample in a stomacher) within 30 min of sampling and incubated at 35°C for 42 h before interpretation for MPNGU per site. MPNGU for 236 carcasses of the 12 monthly evaluations were also determined from pools of 10 samples per carcass. The pools were mixed manually in a stomacher bag by shaking and squeezing. A computer file stored data on MPNGU, carcass number, site, date, line speed, and a code to indicate if carcasses were from cows, had been on the "held" rail, or were subject to visual demerit assessment for the quality-control program. Sample collection and processing and data collection and storage were done by inspection staff at the abattoir.

In addition to the above, a single similar evaluation was conducted on carcasses of cows at the same abattoir from 27 May to 15 June 1992. The cleanliness of the cow slaughter process was measured by the same bacterial counts of 30 carcasses (5 carcasses for each of 6 visits). Cows are afflicted with more pathological changes than younger heifers and steers, which complicates the slaughter and inspection processes. Accordingly, line speed for cows at 165 carcasses per h was slower than for heifers and steers.

**Group evaluation of carcasses in the holding cooler**

The first halves of 17 carcasses were laid out by plant staff, at their discretion, onto a secondary line and samples were collected and data processed as above. At this point carcasses had passed through the water spray-coolers for at least 18 h and had been in the holding cooler, which is without sprayers, for at least 1 h. Two of the 17 carcasses were from cows. The variability of counts at sites of cooled carcasses had not been studied by us and therefore the minimum number of carcasses required for this group carcass evaluation was not known.

**Pathogen identification**

A total of 236 pooled samples from 10 sites for a total of 250 cm$^2$ per carcass of the 12 monthly slaughter process evaluations (see above) were analyzed for the presence of *Listeria monocytogenes* (11). Of each 300-ml pool, 50 ml was transported to the laboratory on ice packs. One-half of each sample (25 ml) was added to 225 ml of listeria enrichment broth (LEB) medium (Oxoid, Unipath Inc., Nepean, Ont.) and incubated for approximately 18 h at 37°C. After mixing, 100 µl aliquots of each LEB-sample mixture were plated onto listeria plating medium (LPM) agar (Difco Laboratories, Detroit, MI) for subsequent polymerase chain reaction (PCR) analysis and were transferred to tubes containing 10 ml of Fraser broth (Oxoid) for subsequent culture isolation (18).

For the isolation of verotoxigenic *E. coli* the second 25 ml of each pool was added to 225 ml of modified Trypticase soy broth (mTSB), incubated for 24 h at 42°C with aeration, and plated on MacConkey agar and sorbital MacConkey agar. The mTSB culture supernatant was tested for verotoxin essentially as previously described (18). However, in this study a 72-h incubation time was used for verotoxin (VT) and VT neutralization assays and toxin identity was confirmed through neutralization with monoclonal antibodies (MAbs). Hybridomas that produced neutralizing MAbs to VT1 and VT2 (hybridomas 13C4 (29) and 11F11 (25) respectively) were obtained from the American Type Culture Collection (Rockville, MD). All non-sorbitol-fermenting verotoxin-positive
isolates were serotyped for the E. coli O157 antigen by slide agglutination using the E. coli O157 Latex Test (Unipath, Nepean, Ontario, Canada). Positive agglutinating colonies were confirmed by biochemical identification and serological testing at the Health Animals Laboratory, Guelph, to be E. coli O157:H7 strains. Coliforms and E. coli were enumerated for each of the 236 carcasses by HGMF technology as described previously (18). Counts of coliforms and E. coli were converted to MPNGU by application of the following formula: MPNGU = N log, [(N / (N - X)) where N is the total number of squares on a filter (N = 1,600), and X is the count of squares containing blue (coliforms) or fluorescent (E. coli) colonies (6).

Statistical analysis
For each of the monthly slaughter floor evaluations and the cooler evaluation, various descriptive statistics (28) were calculated for the log_{10} MPNGU/cm^2 (LMPN) counts. For the LMPN at each site and the average over sites, means, variances, standard errors of the mean and 95% confidence limits for the population mean were obtained. For the data of pooled samples, log_{10} of the most probable number of coliform (LCOL) and E. coli (LEC), similar descriptive statistics were obtained and these variables were correlated. The statistical calculations were carried out using SAS software (26, 27).

RESULTS

Group evaluation of carcasses at the end of the slaughter floor line

Descriptive statistics for the 10 sites of the monthly evaluation with the highest mean are given in Table 1. The LMPN for the sites ranged from 1.14 (thorax) to 2.10 (brisket) and the range of variance was 0.196 (thorax) to 0.484 (rectum). The 95% confidence interval for LMPN for the individual sites averaged about 0.4 log units, except for the rectum site, which had a wider interval.

Descriptive statistics for the site (brisket) with the widest ranges for the means of LMPN (1.42 [April 1994] to 2.23 [August 1994]) and variance (0.174 [February 1995] to 0.636 [August 1994]) over the 12-month study period are given in Table 2.

Descriptive statistics for the means of LMPN over ten sites for all 12 group-carcass evaluations are given in Table 3. The LMPN for the monthly evaluations ranged from 1.11 (April 1994) to 1.62 (July 1994) and the range of variance was 0.023 (April 1994) to 0.084 (June 1994). The 95% confidence interval for LMPN for the monthly evaluations averaged about 0.2 log units. The ratings of slaughter cleanliness are also given in Table 3. These ratings are based on the between-abattoir advisory scale by Mackey and Roberts (22).

Descriptive statistics for the evaluation of carcasses from cows is given in Table 3. Descriptive statistics for the means of LMPN from 236 pooled samples (10 samples per carcass) are given in Table 4. Descriptive statistics for means of log_{10} most probable number of coliforms (LCOL) and of E. coli (LEC) for 236 pooled samples (10 samples per carcass) are given in Table 5.

Group evaluation of carcasses in the holding cooler

The descriptive statistics for the mean of the 10 sites of the single evaluation in the cooler are also given in Table 3.

Associations between most probable number of growth units, coliforms, and E. coli of pooled samples

There were significant positive associations between LMPN and LCOL (r = 0.38, P < 0.001), LMPN and LEC (r = 0.37, P < 0.001) and LCOL and LEC (r = 0.96, P = 0.001). LEC means ranged from 0.20 to 0.65.

Pathogen identification

L. monocytogenes was detected by PCR in 14 of 236 pooled carcass samples. These results were confirmed by culture for 12 of the 14 samples. Verotoxigenic E. coli were detected by PCR and/or Vero cell assay in 6 of 236 pooled carcass samples and confirmed positive by biochemical and serological testing.
mean of log10 most probable number of growth units (MPNGU, hydrophobic grid membrane filter, X)/cm2 averaged over 10 sites (Table I) for each carcass and estimate of log10 CFU (LCFU, standard pour plate method, Y) from the regression equation LCFU = 1.340 + 1.143 LMPN where LMPN is the mean log10 MPNGU (19).

Ratings are based on the mean LCFU (note 95% confidence limits) according to the between-abattoir advisory scale by Mackey and Roberts (22).

Advisory scales for carcasses at the end of the cooler have not been formulated.

by culture for 3 of the 6 samples. One isolate was identified biochemically and confirmed by serotyping to be E. coli O157:H7.

**DISCUSSION**

Both aerobic bacterial counts and E. coli when measured at the end of a process, as applied here for the slaughter and cooling processes, may be used to verify the workings of quality control programs or HACCP systems created for such processes. A high incidence of both types of counts would meet an important criterion for verification of process control. The methods used in this study evolved apart from a HACCP system creation process.

The process of creating such a system may suggest methods of verification other than aerobic bacterial counts.

**TABLE 4. Descriptive statistics for monthly evaluations of MPNGU/cm2 from 10 pooled samples of each of 236 carcasses at the end of the slaughter process**

<table>
<thead>
<tr>
<th>Month</th>
<th>No. of carcasses</th>
<th>Mean</th>
<th>Variance</th>
<th>SEM</th>
<th>log10 MPNGU/cm2</th>
</tr>
</thead>
<tbody>
<tr>
<td>April/94</td>
<td>20</td>
<td>1.43</td>
<td>0.120</td>
<td>0.077</td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>20</td>
<td>1.63</td>
<td>0.207</td>
<td>0.101</td>
<td></td>
</tr>
<tr>
<td>June</td>
<td>20</td>
<td>1.94</td>
<td>0.202</td>
<td>0.101</td>
<td></td>
</tr>
<tr>
<td>July</td>
<td>20</td>
<td>1.97</td>
<td>0.141</td>
<td>0.084</td>
<td></td>
</tr>
<tr>
<td>Aug</td>
<td>20</td>
<td>1.94</td>
<td>0.271</td>
<td>0.116</td>
<td></td>
</tr>
<tr>
<td>Sept</td>
<td>20</td>
<td>1.65</td>
<td>0.218</td>
<td>0.105</td>
<td></td>
</tr>
<tr>
<td>Oct</td>
<td>20</td>
<td>2.09</td>
<td>0.217</td>
<td>0.104</td>
<td></td>
</tr>
<tr>
<td>Nov</td>
<td>20</td>
<td>1.82</td>
<td>0.198</td>
<td>0.100</td>
<td></td>
</tr>
<tr>
<td>Dec</td>
<td>20</td>
<td>1.78</td>
<td>0.297</td>
<td>0.122</td>
<td></td>
</tr>
<tr>
<td>Jan/95</td>
<td>20</td>
<td>2.01</td>
<td>0.116</td>
<td>0.076</td>
<td></td>
</tr>
<tr>
<td>Feb</td>
<td>16</td>
<td>1.65</td>
<td>0.091</td>
<td>0.075</td>
<td></td>
</tr>
<tr>
<td>March</td>
<td>20</td>
<td>1.82</td>
<td>0.158</td>
<td>0.081</td>
<td></td>
</tr>
</tbody>
</table>

*COL, EC: most probable number of coliforms and E. coli,* respectively.

**TABLE 5. Descriptive statistics for monthly evaluations of most probable number of coliforms and Escherichia coli from 10 pooled samples of each of 236 carcasses at the end of the slaughter process**

<table>
<thead>
<tr>
<th>Month</th>
<th>No. of carcasses</th>
<th>log10 (COL/cm2 + 1)</th>
<th>log10 (EC/cm2 + 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April/94</td>
<td>20</td>
<td>0.31 0.128 0.080</td>
<td>0.25 0.125 0.079</td>
</tr>
<tr>
<td>May</td>
<td>20</td>
<td>0.23 0.046 0.048</td>
<td>0.20 0.050 0.050</td>
</tr>
<tr>
<td>June</td>
<td>20</td>
<td>0.54 0.306 0.124</td>
<td>0.46 0.309 0.124</td>
</tr>
<tr>
<td>July</td>
<td>20</td>
<td>0.38 0.135 0.082</td>
<td>0.29 0.154 0.088</td>
</tr>
<tr>
<td>Aug</td>
<td>20</td>
<td>0.57 0.133 0.082</td>
<td>0.46 0.101 0.071</td>
</tr>
<tr>
<td>Sept</td>
<td>20</td>
<td>0.39 0.200 0.100</td>
<td>0.37 0.199 0.100</td>
</tr>
<tr>
<td>Oct</td>
<td>20</td>
<td>0.60 0.262 0.115</td>
<td>0.57 0.239 0.109</td>
</tr>
<tr>
<td>Nov</td>
<td>20</td>
<td>0.35 0.208 0.102</td>
<td>0.27 0.142 0.084</td>
</tr>
<tr>
<td>Dec</td>
<td>20</td>
<td>0.46 0.223 0.106</td>
<td>0.42 0.209 0.102</td>
</tr>
<tr>
<td>Jan/95</td>
<td>20</td>
<td>0.72 0.331 0.129</td>
<td>0.65 0.287 0.120</td>
</tr>
<tr>
<td>Feb</td>
<td>16</td>
<td>0.26 0.086 0.073</td>
<td>0.21 0.056 0.059</td>
</tr>
<tr>
<td>March</td>
<td>20</td>
<td>0.34 0.091 0.061</td>
<td>0.37 0.091 0.062</td>
</tr>
</tbody>
</table>
Assessments of hygienic characteristics of the beef carcass dressing process, for example, may be based on the presence of fecal contamination. E. coli counts are a direct measure of fecal contamination (10) and may thus be used in verifying the efficacy of HACCP systems (8). The high prevalence of E. coli for the 12-month study of the slaughter process of heifers and steers attests to the suitability of this organism for process hygiene verification (Table 5). Similarly, aerobic bacterial counts are a direct measure of microbiological cleanliness. Both aerobic bacteria and E. coli counts are used as indirect measures of the presumptive presence of pathogens. Application of either type of count for HACCP efficacy verification is justified only on the assumption that lower counts of each also imply fewer pathogens see p. 349 in (21). It is acknowledged that at specific sites of a carcass the correlation of aerobic bacteria or E. coli with pathogens is weak (4). For example, in this study both aerobic bacteria and E. coli counts were poor indicators of L. monocytogenes (14 of 236 pools), VTEC (6 of 236 pools) and E. coli O157:H7 (1 of 236 pools). Furthermore there was no association of specific pathogens on carcasses. L. monocytogenes and VTEC were never found in the same pooled 10 samples per carcass. Fortunately, corrective procedures at control points or critical control points which will control aerobic bacteria or E. coli will also control specific pathogens. This facilitates the selection of indicator organisms for HACCP verification.

The low number of L. monocytogenes, VTEC, and E. coli O157:H7 identified is a poor indicator of the prevalence of these pathogens on the carcasses sampled. Bacteria have a heterogeneous distribution on carcasses and only 250 cm² (10 samples, each 25 cm²) of approximately 1,600 cm² of the lateral surface per carcass side were sampled. An unknown larger number of pathogens might be assumed to have been harbored by the carcasses sampled and those not sampled. The number of cases of human illness associated with subsequent generations of pathogens harbored by some 0.6 million carcasses of the sampling period is not known. Nevertheless, the level of human illness was not sufficient to warrant a trace back to the carcasses. The unpredictable and irregular occurrence and frequently low prevalence of any particular pathogen makes the choice of specific pathogens for HACCP verification a dubious exercise. Several pathogens would need to be included in any pathogen-based verification of HACCP systems. Instead, comparative counts, be they aerobic or E. coli, from end-products of processes would indicate the level of control by the quality control program or HACCP system for each process by time and site (Tables 2, 3 and 5). Monitoring by inspection and plant staff of procedures and microbiological records from CCPs would confirm this control.

The aerobic bacterial counts (35°C) for group-carcass evaluations verified the level of control by the plant-created quality control program (1.11 to 1.62 mean log_{10} MPNGU per cm² range for the 12-month period) over the carcass dressing processes for heifers, steers, and cows (Table 3). Comparison of log means requires data with similar variances. The log mean will decrease as the variance increases (21). Aerobic bacterial counts (30 to 32°C) have been previously recommended for HACCP system verification of slaughter processes (23) and the usefulness of such counts to abattoir hygiene assessment has been described (13). In this study samples were taken systematically from 10 predetermined sites, which accommodates the heterogeneous distribution of bacteria on carcasses (12). Regular verification of control of a process permits definition of change over time (Tables 3, 4, and 5) and sampling specific sites of carcasses permits focusing on procedures at CPs or CCPs (Tables 1 and 2). It is noteworthy that the means of log_{10} MPNGU per square centimeter for the monthly evaluations were always higher for pooled samples (n = 20) than for individual samples (n = 200) and the maximum difference was 0.51 (LMPN) for October and November 1994 (Tables 3 and 4). After pooling of the 10 samples for each carcass, the samples and their diluent were shaken manually again, which may account for the increased counts from the pools. This again emphasizes the need for absolute control of method for studies with comparative application within or between plants (19).

The method chosen for HACCP system verification would depend on purpose, convenience, and cost. The speed of method is of minor consideration, as results would only signal the need for corrective action for the process but not acceptance or rejection of the product. This decision is complicated by the vague association of microbiological contamination of the product at the abattoir stage of the food chain with ultimate effects of consumption on human health. Even the association between pathogens and human illness is subject to numerous specific and nonspecific conditions (5).

Aerobic bacterial counts from cooled carcasses are a direct measure of cleanliness for all procedures (slaughter and chill) to that point of carcass production. It is noted that counts on cooled carcasses appear to be comparable to counts on carcasses at the end of the slaughter floor line (Table 3), although the ratio of psychrotrophic to mesophilic bacteria is assumed to have been changed by the cooling process (24). The hygienic adequacy of the cooling process may also be measured by indirect methods, specifically by the more convenient temperature function integration technique (9, 20).

The LMPN data for the slaughter floor and cooler compare favorably with similar data obtained by the same method reported previously for the other high-line-speed abattoir in Alberta (18). For processes in this latter plant, which sells mainly boxed beef, all 12 monthly evaluations for the slaughter floor were rated as “good” (log_{10} aerobic bacterial count (standard plate count) <10⁰ CFU/cm² (22)) and the LMPN for the cooler was 1.22 as compared to 1.40 in this report. The aim of the plant studied in this report is to achieve “excellent” (<10³ CFU/cm²) ratings for the slaughter floor. The motivation is the export of carcasses across international lines, which already prompts extensive trimming of carcasses to achieve a zero-tolerance standard for fecal contamination.

The present work focused on microbiological methods to verify the level of hygienic control of the slaughter and cooling processes in the system of beef production. The
usefulness of aerobic bacterial and E. coli counts to microbiologically measure the hygienic control of carcass production and cooling was demonstrated. The cleanliness of the slaughter process was found to be "excellent" to "good" (22) for all twelve evaluations conducted, and this was achieved with a quality control program which did not include a HACCP system. This does not negate the value of HACCP systems but demonstrates the need of controlling not only CCPs but also CPs of production processes (3). It is concluded that control of bacteria for the processes of beef production may be verified by aerobic bacterial counts as a direct measure of cleanliness or E. coli counts as an indirect measure of fecal contamination. For the latter indicator organism, the sample size and advisory scales would have to be determined for the conditions of cattle and carcass production in Western Canada.

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REFERENCES