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# PRE-WASH BACTERIOLOGICAL EVALUATION OF GROUPS OF BEEF CARCASSES AT SIX ALBERTA ABATTOIRS

K.W.F. JERICHO<sup>1</sup>, J.A. BRADLEY<sup>2</sup> and G.C. KOZUB<sup>3</sup>

- Agriculture Canada, Animal Diseases Research Institute, Alberta, Canada
- Agriculture Canada, Food Production and Inspection Branch,
- Alberta Region, Agriculture Canada, Calgary, Alberta, Canada
  Agriculture Canada Research Station, Agriculture Centre,
  Lethbridge, Alberta, Canada

## INTRODUCTION

Bacteriological contamination of the carcass surface in the abattoir is immediate and unavoidable in the dressing process (Kriaa, 1985). A bacteriological method was developed to measure the level of contamination of beef carcasses (Jericho *et al.*, 1993a). This method was applied in two previous studies which compared heifer and steer carcasses (n=50) and pre and post-wash carcasses (n=26) at the same abattoir (Jericho *et al.*, 1993b; 1993c). Using between and within carcass variance component estimates at carcass sites, we determined that 200 samples from 20 carcasses were required to estimate the mean  $\log_{10}$  Most Probable Number of Growth Units (MPNGU)/cm<sup>2</sup> at a site, within 0.5 units with 95% probability, for group carcass evaluations at this abattoir.

The level of and variation in bacterial contamination at the carcass sites at other abattoirs and, hence, sample size requirements, are not known. Using the above sample size, MPNGU data were obtained from each of six abattoirs operating under federal inspection. These data provided original, albeit time-limited evidence of carcass hygiene, and were used to refine sample size estimates for group carcass evaluations. This is the first report of the evaluation of beef carcass hygiene using statistically determined sample sizes and uniform methodology to estimate mesophilic bacteria at several abattoirs.

## MATERIALS AND METHODS

## Abattoirs

Six beef abattoirs in Alberta were visited between January 27 and June 25, 1992. Two evaluations were done in each during February and March without overlaps in time. Each evaluation was done in four consecutive daily visits (Monday-Thursday). The numbers of carcasses produced per hour on moving lines at the times of evaluation ranged from four to 241/hour (Table 1).

## Animals

The heifers and steers of beef breeds were grain-finished stock from feedlots in Western Canada, received at the abattoirs on the day of slaughter.

#### Carcasses

For each evaluation, five carcasses were sampled on each of four consecutive daily visits to an abattoir. The leading edges of randomly selected carcasses were sampled before the carcass wash station. In abattoirs A-E all but two carcasses came from lots of more than 40 cattle. In abattoir F, carcasses came from lots of 16 carcasses. About 1.5 hours elapsed between the selection of the first and fifth carcass at any visit. The percent of carcasses sampled relative to production volume during the sampling period at each abattoir ranged from 1.3-83.3% (Table 1). The distribution of carcasses by sex is given in Table 1.

#### Samples

One excision sample (5x5x0.5cm) was taken for bacteriological study by methods and carcass at sites (hock, rump lateral, rump medial, rectum, flank, thorax, brisket, axilla, shank, and neck) that have been as previously described (Jericho *et al.*, 1993a). Site selection was based on the distribution of visual demerits over the lateral surfaces of carcasses (Jericho *et al.*, 1993a). Samples were placed individually in stomacher bags, transported in a cooler  $(4 \pm 1^{\circ}C)$  to the laboratory, and processed three to seven hours (abattoir D) after sampling.

#### Microbiology

The MPNGU of aerobic bacteria on hydrophobic grid membrane filters (HGMF)(ISO Grid, QA Life Sciences, San Diago, Cal 92121) were enumerated for each sample as previously described (Jericho *et al.*, 1993a; 1993b; 1993c). The HGMFs were incubated (35°C) on tryptic soy agar plates with 0.01% of 2,3,5 triphenyltetrazolium chloride (TTC, Sigma Chemicals Co., T8877) for 42 hours. The MPNGU were assessed by an automated HGMF interpreter (MI100 HGMF Interpreter System, Richard Brancker Research LTD., 27 Monk St. Ottawa, Ontario, K1S 3 Y7) and a computer data file of results and associated variables such as lot number, date, sex, line speed, time of carcass processing etc. was created.

#### Statistical analyses

Using log<sub>10</sub> MPNGU/cm<sup>2</sup> (LMPN) transformed bacterial counts, various summary statistics (the mean, standard error of the mean and 95% confidence limits for the mean) were determined for each site on the carcasses within an abattoir (Snedecor and Cochran, 1980). For each site, estimates of the between carcass variance components for the abattoirs were tested for homogeneity using Bartlett's test of homogeneity of variance. A pooled estimate of variance was subsequently obtained. The between carcass variance was used to determine the number of carcasses required to estimate the mean LMPN at a site.

Analyses of variance (Snedecor and Cochran, 1980) of the LMPN for each abattoir were carried out to determine if there were effects due to day of slaughter or sex, and if there were interaction of any such effects with the carcass site. A split-plot statistical model was used, with effects due to day or sex being included in the whole-plot part of the analysis and effects of site and the day or sex x site interaction being in the subplot analysis. A similar analysis was carried out over the abattoirs to determine if there was an abattoir x site interaction. The LMPN means for the sites were clustered (Scott and Knott, 1974) within each abattoir to obtain approximate groupings of sites with similar LMPN levels. The UNIVARIATE, VARCOMP and GLM procedures of the SAS software (SAS Institute, 1989; 1990) were used in the statistical analyses.

#### **RESULTS AND DISCUSSION**

Means of counts per site and over sites

The mean LMPN per site for each abattoir and the standard errors (SE), are given in Table 2. The half-lengths of the confidence intervals for the means ranged from 0.14 to 0.41 and reached the upper limit only for one site at three abattoirs. The mean LMPN over sites for each of the six abattoirs ranged from 1.52 to 2.64 (Table 2).

### Variability in counts at sites and sample size estimation

The between carcass variance components for each site were generally homogenous among the abattoirs. The average within abattoir variance is given for each site in Table 3. Using these variance estimates, the numbers of carcasses required to estimate the mean LMPN within L units with 95% probability when one sample is taken at a site are indicated in Table 3.

## Effect of site on counts

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The analyses of variance over abattoirs indicated that there was a highly significant site x abattoir interaction (P < 0.001). This reflected the lack of consistency from abattoir to abattoir in differences in LMPN among sites. The sites were clustered into four homogenous groups (a-d) in abattoirs B and F but only two groups (a,b) in abattoirs A and C (Table 2). Only one site (brisket) belonged to the same group (a) in all six abattoirs.

## Effect of day of slaughter and sex on LMPN

Analyses of variance of the LMPN data for each abattoir over the sites indicated that the effect of site was highly significant (P<0.001), but the effect of day of slaughter and the day x site interaction was not significant (P>0.05). Similarly, the effect of sex and the sex x site interaction was not significant.

Our objective is to develop bacteriological procedures that can be used to monitor carcass hygiene as a measure of process control. This requires the examination of individual carcass sites as well as an overall carcass assessment. Although ten sites per carcass have been used in this study to measure control over hygiene, it may be sufficient to determine the mean log MPNGU/cm of fewer sites based on cluster analysis. On the other hand, the clustering of sites may be inconsistent over time, and thus estimates from all 10 sites may be useful in process control, signalling the possibility of improper processing at certain quality control points.

The definition of the factors which lead to the levels of contamination was not at issue. Therefore, a description of each abattoir was considered superfluous. Furthermore, the different sources of cattle processed at different times at the abattoirs ruled out comparisons between abattoirs. The lack of effect of day of slaughter and sex on counts supports the results of a previous study at abattoir A (Jericho *et al.*, 1993b). Based on limited data of this study it appears that there is no linear relationship between line speed and LMPN (P>0.05).

The sample size used for this study was derived from estimates of LMPN variation at sites of carcasses from one abattoir as previously described (Jericho *et al.*, 1993a; 1993b; 1993c). Estimates of the mean within 0.5 log units was chosen as the desired level of precision. In this study the half-length of the confidence interval for the LMPN was less than 0.42 for all the sites at all the abattoirs. The sample size of 20 carcasses (one sample at each of ten sites) was therefore more than adequate for the variability encountered at the six abattoirs and the level of precision stipulated. Increasing the level of control over the processes of carcass production should decrease the variation in bacterial contamination at carcass sites and thus reduce the required sample size. The refined sample size of this study would therefore exceed the requirements for more controlled conditions. Additional data from more controlled conditions over time will permit further refinement of the sample size estimate and investigation of the site x time interaction. Collection of these data is in progress at abattoirs A and B. Reduction in the number of carcass sites for group carcass evaluation, levels of precision and their effect on sample size are the subjects of a further report. A thorough examination of the significance and variability of carcass contamination over time must be made before rational acceptability levels can be discussed and implemented in quality control programs.

#### CONCLUSION

The sample size determined from the average within abattoir variation at a site assured that sampling 10 sites/20 carcasses was more than adequate for these group-carcass evaluations. The means of the  $\log_{10}$  counts/cm<sup>2</sup> for carcass sites at each abattoir were clustered into 2-4 homogenous groups. The means for the abattoirs ranged from 1.52 - 2.64 and were without a linear relationship to line speed.

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Table 1. Carcasses produced and sampled at each abattoir.

Abattoir	Heifer/steer Carcasses	Line Speed/h	% Carcasses Sampled <sup>b</sup> 1.3	
A	8/12	241		
В	3/17	207	1.6	
С	9/11	115	2.9	
D	2/18	90	3.4	
Е	6/14	45	7.3	
F	16/2/2ª	4	83.3	

<sup>a</sup> Abattoir F included two bulls. <sup>b</sup> On each of four daily visits five carcasses were sampled randomly from the carcass population that was on-line during the 1.5h sampling period.

	Abattoir		
Site	A Mean SE	B Mean SE	C Mean SE
н	1 032° 0 148	1,660b 0,155	2 3542 0 165
T	1.992a 0.120	1.755b 0.103	2.334a 0.105
M	2 260a 0 159	2.025b 0.135	2.6159 0.129
R	1.935a 0.128	1 994b 0 159	2.015a 0.125
F	1 340b 0 095	1 397c 0 186	1.885b 0.175
T	1.519b 0.159	0.858d 0.153	1.852b 0.148
В	2.274a 0.152	2.318a 0.140	2.334a 0.191
A	1.700b 0.152	2.470a 0.181	1.620b 0.184
S	1.678b 0.194	1.219c 0.162	1.586b 0.142
N	1.549b 0.189	0.856d 0.147	1.341b 0.117
Over sites	1.808 0.052	1.655 0.072	2.002 0.059
	Abattoir		
Site	Abattoir D Mean SE	E Mean SE	F Mean SE
Site H	Abattoir D Mean SE 1.355b 0.195	E Mean SE 1.561b 0.184	F Mean SE 2.694b 0.118
Site H L	Abattoir D Mean SE 1.355b 0.195 2.576a 0.155	E Mean SE 1.561b 0.184 2.075a 0.154	F Mean SE 2.694b 0.118 2.693b 0.099
Site H L M	Abattoir D Mean SE 1.355b 0.195 2.576a 0.155 2.662a 0.112	E Mean SE 1.561b 0.184 2.075a 0.154 2.224a 0.129	F Mean SE 2.694b 0.118 2.693b 0.099 3.134a 0.077
Site H L M R	Abattoir        D        Mean      SE        1.355b      0.195        2.576a      0.155        2.662a      0.112        1.631b      0.206	E Mean SE 1.561b 0.184 2.075a 0.154 2.224a 0.129 1.506b 0.164	F Mean SE 2.694b 0.118 2.693b 0.099 3.134a 0.077 2.463c 0.145
Site H L M R F	Abattoir      D      Mean    SE      1.355b    0.195      2.576a    0.155      2.662a    0.112      1.631b    0.206      1.400b    0.168	E Mean SE 1.561b 0.184 2.075a 0.154 2.224a 0.129 1.506b 0.164 1.187c 0.131	F Mean SE 2.694b 0.118 2.693b 0.099 3.134a 0.077 2.463c 0.145 2.619b 0.129
Site H L M R F T	Abattoir      D      Mean    SE      1.355b    0.195      2.576a    0.155      2.662a    0.112      1.631b    0.206      1.400b    0.168      0.918c    0.169	E Mean SE 1.561b 0.184 2.075a 0.154 2.224a 0.129 1.506b 0.164 1.187c 0.131 0.865c 0.189	F      Mean    SE      2.694b    0.118      2.693b    0.099      3.134a    0.077      2.463c    0.145      2.619b    0.129      2.204d    0.200
Site H L M R F T B	Abattoir      D      Mean    SE      1.355b    0.195      2.576a    0.155      2.662a    0.112      1.631b    0.206      1.400b    0.168      0.918c    0.169      2.352a    0.194	E Mean SE 1.561b 0.184 2.075a 0.154 2.224a 0.129 1.506b 0.164 1.187c 0.131 0.865c 0.189 2.244a 0.139	F      Mean    SE      2.694b    0.118      2.693b    0.099      3.134a    0.077      2.463c    0.145      2.619b    0.129      2.204d    0.200      3.226a    0.094
Site H L M R F T B A	Abattoir      D      Mean    SE      1.355b    0.195      2.576a    0.155      2.662a    0.112      1.631b    0.206      1.400b    0.168      0.918c    0.169      2.352a    0.194      2.864a    0.128	E Mean SE 1.561b 0.184 2.075a 0.154 2.224a 0.129 1.506b 0.164 1.187c 0.131 0.865c 0.189 2.244a 0.139 1.402b 0.128	F      Mean    SE      2.694b    0.118      2.693b    0.099      3.134a    0.077      2.463c    0.145      2.619b    0.129      2.204d    0.200      3.226a    0.094      2.914b    0.097
Site H L M R F T B A S	Abattoir      D      Mean    SE      1.355b    0.195      2.576a    0.155      2.662a    0.112      1.631b    0.206      1.400b    0.168      0.918c    0.169      2.352a    0.194      2.864a    0.128      2.239a    0.182	E Mean SE 1.561b 0.184 2.075a 0.154 2.224a 0.129 1.506b 0.164 1.187c 0.131 0.865c 0.189 2.244a 0.139 1.402b 0.128 1.170c 0.208	F      Mean    SE      2.694b    0.118      2.693b    0.099      3.134a    0.077      2.463c    0.145      2.619b    0.129      2.204d    0.200      3.226a    0.094      2.914b    0.097      2.465c    0.162
Site H L M R F T B A S N	Abattoir      D      Mean    SE      1.355b    0.195      2.576a    0.155      2.662a    0.112      1.631b    0.206      1.400b    0.168      0.918c    0.169      2.352a    0.194      2.864a    0.128      2.239a    0.182      1.699b    0.174	E Mean SE 1.561b 0.184 2.075a 0.154 2.224a 0.129 1.506b 0.164 1.187c 0.131 0.865c 0.189 2.244a 0.139 1.402b 0.128 1.170c 0.208 1.000c 0.176	F      Mean    SE      2.694b    0.118      2.693b    0.099      3.134a    0.077      2.463c    0.145      2.619b    0.129      2.204d    0.200      3.226a    0.094      2.914b    0.097      2.465c    0.162

Table 2. Means of log<sub>10</sub> MPNGU/cm<sup>2</sup> and Standard Errors (SE) for carcass sites at each abattoir<sup>a</sup>.

\* Comparison between plants is not indicated because cattle were derived from different sources and evaluations were done at different times.

<sup>b</sup> Means followed by the same letter within a column indicate sites that are homogenous according to cluster analysis

at the 5% significance level.

~	Between carcass	# of car	# of carcasses <sup>b</sup> when L=			
Site	variance mean <sup>a</sup>	0.5	0.4	0.3	0.2	
H	0.4865 2.028	8	13	22	49	
L	0.3261 2.241	6	9	15	33	
M	0.3010 2.511	5	8	14	31	
R	0.4007 1.979	7	11	18	41	
F	0.4264 1.726	7	11	19	43	
T	0.5652 1.361	10	15	26	57	
В	0.3854 2.594	7	10	18	39	
A	0.4790 2.143	8	12	22	48	
S	0.6558 1.872	11	17	30	66	
N	0.5124 1.569	9	13	23	52	

Table 3. Estimates of between carcass variance at a site and the number of carcasses required to estimate log10 MPNGU/cm<sup>2</sup> with a desired degree of precision.

<sup>a</sup> Average within abattoir variation (188 df) in log<sub>10</sub> units (mean log<sub>10</sub> MPNGU/cm<sup>2</sup> in parentheses). <sup>b</sup> Number of carcasses required to estimate the mean log<sub>10</sub> MPNGU/cm<sup>2</sup> within L units with 95% probability when one sample is taken at a site.