MICROBIOLOGICAL BASELINE SURVEY OF RAW BEEF CARCASSES IN ONTARIO ABATTOIRS.

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Introduction

During a review of the food safety system in Ontario, the Ministry of Agriculture and Food (OMAF) recognised the need to update its standards to keep pace with changes in science, technology, national and international standards, consumer lifestyle and industry practices. OMAF has taken the lead in improving Ontario's food safety system by designing and implementing a field-to-fork, science-based food safety system. As part of the continuous review of the inspection programs, looking for ways to improve, one step is to conduct baseline studies to determine the prevalence of microbiological, physical and chemical hazards associated with some foods.

The United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS), conducted a nationwide survey of steers and heifers during 1992-3 and one of cows and bulls during 1993-4. These studies sampled 2089 steers and heifers and 2112 cows and bulls for the presence of *Clostridium perfringens*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni/coli*, *Salmonella* spp. and *Escherichia coli* 0157:H7. The survey also examined indicator organisms (total coliforms, *E. coli* (Biotype I) and Aerobic Plate Counts (APC) at 35°C) (4,5).

In Australia, a study was conducted to determine the microbiological quality of beef carcasses processed at slaughter establishments that were selected to statistically represent the beef carcass industry in the country. Samples were collected from 1063 beef carcasses at slaughter establishments that target both Australian domestic and export markets. Samples were analysed for the presence of indicator organisms such as Aerobic Plate Count, total coliforms and *E.coli* (biotype I). In addition, the presence of pathogens such as *Salmonella* spp., *Listeria* spp., *Campylobacter* spp., *Staphylococcus* spp., and *E.coli* O157:H7 were examined (6).

In 1998, the Food Inspection Branch, OMAF, evaluated the need for microbial baseline studies in meat products in provincially licensed abattoirs, and determined that microbial baseline studies were required for three species based on their contribution to the total slaughter volume in the 1997 calendar year: chickens (40.3 million kg); pork (37.7 million kg) and beef (31.4 million kg). (1) These baseline studies were intended to establish a clear picture of the range and distribution of a variety of pathogenic bacteria to determine which organisms present the greatest risk. In addition, indicator organisms were included in the studies to provide an assessment of the extent of faecal contamination and/or poor hygienic practices.

A previous microbial baseline study of pork conducted by the Food Inspection Branch showed that microbial prevalence varies based on slaughter volume (3). Therefore the branch was interested in examining whether the same effect was true in beef.

Objectives of Study

The objectives of the microbial baseline study for beef were to:

1) determine the prevalence of specific pathogens and levels of indicator organisms and/or pathogens in the targeted populations of fed beef (steers and heifers) and culled beef (cows and bulls);
2) provide data to allow the meat inspection program to target and prioritize its resources to those areas, processes or products which warrant

the most urgent intervention activities. In particular, the branch wished to examine the impact of processing volume and dressing practices, including dressing method, dehiding process and shrouding.
3) provide data to serve as a baseline against which to measure the impact of intervention programs such as HACCP or regulations.

Materials and Methods

Provincially licensed plants tend to be small in size, and vary in the volume of beef processed (Table 1). Nearly all plants that process red meat process some beef, resulting in a very large number of plants with a very small processing volume. A stratified design therefore allowed more effective sampling of these lower volume plants. Without a stratified design, sampling by volume would have resulted in a majority of samples originating from the "high" volume plants.

Table 1. Distribution of beef processing among licensed plants

STRATUM	VOLUME (head/yr)	# PLANTS	% TOTAL PLANTS	% TOTAL VOLUME
High	> 1039	10	5.6	53.1
Medium	520 - 1039	26	14.7	16.8
Low	1 - 519	141	79.7	30.1

Sampling frequency was based on probabilities proportional to volume within each stratum. Plants & carcasses were selected randomly on sampling days. The study was conducted over a 12 month period to account for seasonal variation, with full geographic representation of the province to account for regional variations. The target population included steers, heifers, cows and bulls. The sample size chosen was based on variances found in previous studies of pathogens and indicator organisms. Collection of 522 samples per stratum was planned, for an overall total of 1566 samples.

Inspectors were trained in aseptic sampling techniques and the study methodology (2). At the plant, carcass selection was carried out by the inspector, using a random number table. Sampling was carried out using a sponge sampling method 12-36 hours post-slaughter. Buffered Peptone Water was used as the diluent. Three sampling sites were chosen per carcass (brisket, flank and rump) using a 100 cm^2 template. Sampling kits were provided by International Bioproducts. Information collected at the time of sampling included type of beef (fed vs. culled); beef class (steer, heifer, cow or bull); dressing method (bed or rail); dehiding method (manual or mechanical) and shrouding practices. Information about the samples was recorded and transmitted electronically to the Laboratory Services Division, University of Guelph (LSD). Samples were packed in chilled shipping containers, time and date stamped, and shipped to the laboratory using a commercial courier. A strict set of sample acceptance/rejection criteria was implemented. Only samples which were received by the laboratory within 24 hours of collection, complete with the three swabs, uncontaminated, non-leaking and whose temperature was between 0° and 8°C were accepted for microbial analysis.

Pooled swabs from 1459 carcasses were analyzed for: aerobic colony count (ACC), total coliform count (TCC), *Escherichia coli* count (ECC), *Campylobacter* spp., *Listeria monocytogenes*, *Salmonella* spp. and verotoxigenic E. coli (VTEC). The analytical methods used for *E. coli* count, *Salmonella*, *Listeria monocytogenes*, and aerobic plate count were those accredited by the CFIA and de-

scribed by Health Canada, HPB Compendium of Analytical Methods (7,8,9). The *C. jejuni/coli* analytical method and the verocell assay for *E. coli* 0157:H7 were modifications of those described in the former Agriculture and Agri-Food Canada laboratory procedure manual. Serotyping of *Campylobacter* and *Salmonella* isolates was provided by participating Health Canada Laboratories in Guelph and Winnipeg.

Results were analyzed with a statistical analysis system (SAS Inc. Cary, N.C) for associations with strata, geographical location, season and operational variables.

Results and Discussion

The results are presented in tables appended to this article. Table 2 demonstrates the prevalence of microorganisms on raw beef carcass samples. Table 3 presents the impact of production volume on the prevalence of microorganisms on the surfaces of raw beef carcasses sampled. Table 4 shows the impact of production volume on means of indicator organisms per cm^2 on raw beef carcass surface samples. Tables 5-9 illustrate the impacts of beef type, beef class, dressing method, de-hiding method and shrouding on the prevalence of microorganisms on raw beef carcass surface samples. A short summary of the results obtained in the study follows.

Aerobic bacteria were recovered from the surface of all (100%) the 1454 beef carcasses tested in the study (Table 2). Coliform bacteria were recovered from 27.8% of 1459 carcasses and *E.coli* (Biotype 1) was recovered from 18.6% of 1459 carcasses. *L.monocytogenes* was the most common pathogen, recovered from 9.9% of 1459 carcasses, followed by *Salmonella*, which was recovered from 1.6% of 1459 carcasses, *Campylobacter jejuni/coli,* recovered from 1.5% of 1444 carcasses and VTEC, recovered from 0.3% of 1458 carcasses. None of the VTEC isolates was *E.coli* O157:H7.

The impact of production volume on the prevalence of microorganisms on beef carcasses tested in this study is presented in Table 3. Total coliforms, *Salmonella* and *Campylobacter* were significantly lower on carcasses from low and medium volume plants than from high volume plants, but there was no significant difference in *L.monocytogenes* or VTEC. On the other hand, production volume had little impact on the counts of indicator organisms (Table 4) with the exception that *E.coli* counts were significantly higher in medium volume plants than in high or low volume plants.

The impacts of beef type and beef class on the prevalence of microorganisms on raw beef carcasses are presented in Tables 5 and 6. Culled beef were significantly higher in the prevalence of total coliforms, *E.coli, Salmonella and Campylobacter* than fed beef. Heifers were significantly lower in incidence of total coliforms and *E.coli* than the other beef classes. Cows were significantly lower in prevalence of *L.monocytogenes* but significantly higher in prevalence of *Salmonella* and *Campylobacter.*

Dressing method had a significant impact on the prevalence of some microorganisms (Table 7). Rail dressing resulted in a significantly higher incidence of total coliforms, *E.coli* and *L.monocytogenes* as compared to bed dressing. The de-hiding method also impacted prevalence (Table 8), with mechanical de-hiding resulting in significantly higher prevalence of total coliforms, *E.coli*, and *L.monocytogenes*. Shrouding did not affect the prevalence of microorganisms on the beef carcasses included in the study (Table 9).

The results of the current study could not be compared directly with the results from the FSIS baseline study due to differences in sampling methodology. FSIS data was based on analyses of excised samples while our data was obtained by sampling with sponges. Nevertheless, when comparing data obtained for fed beef in both studies some comparisons can be noted: the overall prevalence of *L. monocytogenes* was higher in the OMAF study (10.2%) compared to the US study (4.1%). On the

other hand, prevalence of *Campylobacter jejuni/coli* in the OMAF study (1.2 %) was lower compared to the US study (4.0%). However, both studies were close in terms of prevalence of *Salmonella* (1.3% in OMAF study versus 1.0% in US study) and VTEC (0.3% in OMAF study versus 0.2% in US study).

The results for culled beef can be compared as follows: the prevalence of *L.monocytogenes* in the OMAF study (7.9%) was lower compared to the US study (11.3%). However, the prevalence of *Salmonella* and *Campylobacter jejuni/coli* in the OMAF study (4.2% and 4.3% respectively) were higher than the US study (2.7% and 1.1% respectively) for this class of animals. VTEC was not recovered from culled beef in either the OMAF or US study.

Similar to the US study, the Australian study was based on analyses of excised samples as opposed to OMAF data obtained by sampling with sponges. Keeping in mind the differences in sampling methodology in the studies, the prevalence of microorganisms on carcasses processed for the Australian domestic market compares to the OMAF study as follows: total coliforms 46.5% (OMAF:27.8%), *E.coli* (biotype I) 27.1% (OMAF:18.6%), *Salmonella* 1.4% (OMAF: 1.6%), *Campylobacter* 0.81% (OMAF:1.5%) and *L.monocytogenes/coli* 15% (OMAF: only *L.monocytogenes,* 9.9%). *E.coli* O157:H7 was not detected in either the OMAF study or the part of the Australian study that targeted their domestic market.

Conclusions

From the present study it would appear that the overall microbiological quality of beef carcasses processed in Ontario's provincially licensed abattoirs was similar to that of carcasses produced in other jurisdictions such as the United States and Australia.

Both rail dressing and mechanical de-hiding resulted in significantly higher incidences of total coliforms, *E.coli* and *L.monocytogenes* compared to bed dressing and manual de-hiding respectively. Bed dressing and manual de-hiding are mainly used in lower volume plants as opposed to rail dressing and mechanical de-hiding. As a result, and taking into account the impacts of rail dressing and mechanical de-hiding mentioned above, the prevalence of coliforms and counts of generic *E.coli* were significantly lower in the low volume plants as compared to the higher volume plants. Furthermore, lower volume plants had significantly lower incidences of *Salmonella* and *Campylobacter* compared to the higher volume plants.

Heifers had significantly lower total coliform and *E.coli* counts than other beef classes. However, contamination of cow carcasses with *Salmonella* and *Campylobacter* was significantly higher compared to carcasses of bulls, heifers and steers.

Implications

Although it is not valid to predict the safety of meat products based on counts of indicator microorganisms such as coliform counts, or *E.coli* counts, such counts may be an indicator of increased probability for presence of pathogens. The prevalence of coliforms and counts of generic *E.coli* were significantly lower in the lower volume plants compared to the higher volume plants.

The results obtained for the low volume plants possibly reflect the influence of a slower processing rate. Potentially pathogenic bacteria reside on the hide or in the intestinal tract of the animals slaughtered. However, slowing down the processing rate (the speed of the line) may not be an option to reduce carcass contamination. The currently available dressing procedures can not be relied upon to prevent or remove all of the bacterial contamination on the carcass surface. Therefore, attention should be paid to evaluate intervention strategies that will improve the microbiological quality and safety of meat.

Ontario is developing a new regulation for meat inspection based on the National Meat and Poultry Regulation and Code. Once the new regulation under the new *Food Safety and Quality Act, 2001* (not yet in force) is in place, beef slaughtering

plants will need to establish process control programs (e.g., decontamination hurdles, HACCP) for reducing microbiological contamination of carcasses. This study suggests that smaller, less automated abattoirs are capable of achieving acceptable microbial performance standards.

References

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Table 2. Prevalence of microorganisms on raw beef carcass surface samples

MICROORGANISMS		# SAMPLES	# POSITIVE	% POSITIVE
INDICATORS	*ACC*	1454	1454	100.0
	TCC	1459	405	27.8
	ECC	1459	271	18.6
PATHOGENS	L. monocytogenes	1459	145	9.9
	Salmonella spp.	1459	24	1.6
	C. jejuni/coli	1444	22	1.5
	VTEC	1458	5	0.3

Table 3. Impact of production volume on the prevalence[1] of microorganisms on raw beef carcass surface samples

MICROORGANISMS[2]		HIGH VOLUME	MEDIUM VOLUME	LOW VOLUME
INDICATORS	TCC**	32.1	25.2	24.8
	ECC[ns]	19.5	19.6	16.4
PATHOGENS	L. monocytogenes[ns]	7.9	10.5	12.1
	Salmonella spp.***	3.3	0.2	0.9
	C. jejuni/coli*	2.5	0.5	1.4
	VTEC[ns]	0.4	0.0	0.7

[1]Data values are in % positive
[2]Statistical Probabilities: [ns] = not significant; * = P<.05; ** = P<.01, *** = P<.001

Table 4. Impact of production volume on means[1] of indicator organisms per cm^2 on raw beef carcass surface samples

MICROORGANISMS[2]		*HIGH* (570)[3] MEAN	SE[4]	MEDIUM (448) MEAN	SE[4]	LOW (436) MEAN	SE[4]
INDICATORS							
	ACC[ns]	4.20	0.04	4.12	0.04	4.07	0.04
	TCC[ns]	1.65	0.09	1.93	0.11	1.72	0.11
	ECC*	1.62[a]	0.11	1.95[b]	0.13	1.54[a]	0.14

[1]Mean values are in $\log_{10}$ cfu/cm^2
[2]Statistical Probabilities: [ns] = not significant; * = P<.05; ** = P<.01, *** = P<.001
[3](n) = Number of samples
[4] SE = Standard Error of the mean
[a,b] Means within the same row with different superscripts differ significantly

Table 5. Impact of beef type on the prevalence[1] of microorganisms on raw beef carcass surface samples

MICROORGANISMS[2]		CULLED BEEF	FED BEEF
INDICATORS	TCC**	36.51	26.72
	ECC*	24.87	17.92
PATHOGENS	L. monocytogenes[ns]	7.94	10.17
	Salmonella spp.**	4.23	1.29
	C. jejuni/coli**	4.30	1.22
	VTEC[ns]	0.00	0.32

[1]Data values are in % positive
[2]Statistical Probabilities: [ns] = not significant; * = P<.05; ** = P<.01, *** = P<.001

Table 6. Impact of beef class on the prevalence[1] of microorganisms on raw beef carcass surface samples

MICROORGANISMS[2]		BULL	COW	HEIFER	STEER
INDICATORS	TCC***	37.89	35.11	21.90	31.08
	ECC***	23.16	26.60	13.07	22.31
PATHOGENS	L. monocytogenes*	12.63	3.19	8.83	11.38
	Salmonella spp.***	1.05	7.45	1.02	1.54
	C. jejuni/coli***	1.09	7.45	1.20	1.24
	VTEC[ns]	0.00	0.00	0.51	0.15

[1]Data values are in % positive
[2]Statistical Probabilities: [ns] = not significant; * = P<.05; ** = P<.01, *** = P<.001

Table 7. Impact of dressing method on the prevalence[1] of microorganisms on raw beef carcass surface samples

MICROORGANISMS[2]		BED	RAIL
INDICATORS	TCC ***	24.7	40.82
	ECC **	17.48	24.15
PATHOGENS	L. monocytogenes ***	8.43	14.63
	Salmonella spp. ns	1.74	1.36
	C. jejuni/coli ns	1.67	1.03
	VTEC ns	0.26	0.34

[1]Data values are in % positive
[2]Statistical Probabilities: ns = not significant; * = P<.05; ** = P<.01, *** = P<.001

Table 8. Impact of dehiding on the prevalence[1] of microorganisms on raw beef carcass surface samples

MICROORGANISMS[2]		MANUAL	MECHANICAL
INDICATORS	TCC***	24.63	42.96
	ECC***	17.09	27.08
PATHOGENS	L. monocytogenes**	8.85	13.72
	Salmonella spp.ns	1.82	1.08
	C. jejuni/coli ns	1.75	0.73
	VTEC ns	0.26	0.36

[1]Data values are in % positive
[2]Statistical Probabilities of Significance: ns = not significant; * = P<.05; ** = P<.01, *** = P<.001

Table 9. Impact of shrouding on the prevalence[1] of microorganisms on raw beef carcass surface samples

MICROORGANISMS[2]			SHROUD	NO SHROUD
INDICATORS		TCC [ns]	29.74	27.29
		ECC [ns]	18.47	19.01
PATHOGENS		L. monocytogenes.[ns]	9.11	9.94
		Salmonella spp.[ns]	2.16	1.46
		Campylobacter jejuni/ coli [ns]	2.40	1.19
		VTEC[ns]	0.24	0.29

[1]Data values are in % positive
[2]Statistical Probabilities of Significance: [ns] = not significant