Sampling Beef Carcasses for *Escherichia coli*

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Background

In order to effectively validate and verify HACCP protocols that are implemented in meat processing establishments, sampling and testing procedures must be identified that are robust, practical, accurate and reproducible. In particular, it is essential that sampling techniques provide an accurate representation of the level of microbial contamination on product at critical points during processing. The USDA FSIS has mandated a regime for sampling carcasses destined for the USA market.

Objectives

Investigate aspects of the USDA FSIS Pathogen Reduction Scheme (MegaRegs) sampling methods including (1) variation in recovery from sponge compared to excision sampling; (2) variation in sampling 2 versus 3 sites on the carcass; (3) variation in *E. coli* counts by different culture methods; (4) differences in recovery of bacteria by different operators.

Methods

Objectives 1-3

A. Sampling. Beef carcasses were sampled (3x100cm^2) by both excision and swabbing at a Victorian domestic abattoir. Each carcass was sampled by sponging (USDA) at rump, flank and brisket, then the same area excised. Sponge samples were stored in Whirlpak bags in Butterfield's solution and excised samples were folded inwards and stored in sterile bags. Samples were returned at 4°C to the laboratory and tested on arrival.

B. Sample Preparation. Both sponge and excised samples were stomached for 2 minutes and the fluid expelled from the bag, diluted in peptone water and examined for total viable counts (TVC) and *E. coli*.

C. Microbiological Analysis. Samples were analysed for TVC by AS 1766.1.3 (1991) and for *E. coli* by both Petrifilm and Most Probable Number (MPN: AS 1766.2.3, 1992). In brief:

a. For TVC, diluted aliquots were plated in pour plates (Plate Count Agar: Oxoid) and incubated at 25°C for 48h.

b. For *E .coli* counts by Petrifilm, 1ml aliquots were plated onto Petrifilm and incubated at 35°C for 48h.

c. For *E. coli* by MPN, 1 ml aliquots of the original suspension and dilutions of that were added to a total of 15 lauryl tryptose broth tubes in accordance with AS 1766.2.3.

Objective 4 (Operator variation)

Five operators (3 experienced laboratory technicians and 2 quality assurance officers with limited experience) sampled 10 carcasses each (composite 3x100cm^2 samples as described above) on each of 5 days at the same abattoir. Samples were evaluated for *E. coli* and coliforms by Petrifilm as described above.

Results and Discussion

1. Comparison of excision versus sponge-sampling in recovery of *E. coli*.

Excision resulted in higher recovery rates compared with sponging (Table 1). The data is presented as a frequency distribution to allow for comparison with MegaRegs 3 class sampling plan where m = 5, M = 100, c=3.

	Petrifilm			MPN		
E .coli/cm^2	Rump (E,S)*	Flank (E,S)	Brisket (E,S)	Rump (E,S)	Flank (E,S)	Brisket (E,S)
Not detected	64, 89	53, 85	91, 94	51, 88	36, 69	65, 86
<1	9, 5	23, 13	3, 8	17, 7	30, 26	27, 15
1-5	15, 4	19, 2	6, 0	18, 3,	29, 6	9, 1
5-100	13,3	6, 1	2, 0	12, 4	4, 1	1, 0
>100	1, 1	1, 1	0, 0	4, 0	3, 0	0, 0
% positive	Petrifilm (E,S)	33, 13		MPN (E,S)	51, 21	

*E = excised sample, S = sponge sample

Table 1. Frequency distribution of *E. coli* counts/cm^2 from different sites on beef carcasses and estimated by different methods

2. Comparison of 3 versus 2 site sampling for evaluation of microbial status of carcasses.

The mean TVC at each site for sponge samples is shown in Figure 1. Total viable counts are similar at each site, with counts at flank and brisket marginally higher than rump. However the *E. coli* data (Table 2) indicate that the recovery of the indicator organism recommended in the MegaRegs is higher in rump than brisket or flank. Elimination of the rump from the sample sites may result in a lower *E. coli* count for the carcass as a whole than if all 3 sites were used.

3. Comparison of Petrifilm with Most Probable Number (MPN) for enumeration of *E. coli*.

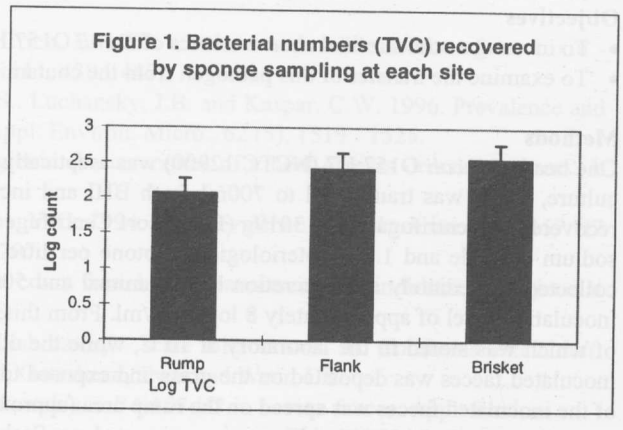
See Table 1 for data presented as a frequency distribution. Table 2 presents the data as arithmetic counts of *E .coli*/cm^2. The estimation of *E .coli* by MPN resulted in higher counts on excised samples compared to sponge samples. However the standard errors for these means are large and there is no statistically significant difference. There were no significant differences between the methods for estimation of *E. coli* numbers (Table 2). However, if the data is considered as a frequency distribution (Table 1), there would be differences in compliance with the MegaRegs sampling plan. When samples were considered as composites for each carcass, estimation by the Petrifilmmethod resulted in 2 carcasses above the 10^2 upper limit, while none were in that range by the MPN method. In the marginal range of 5-10^2/cm^2, only 1 carcass was positive by MPN that was not positive by Petrifilm.

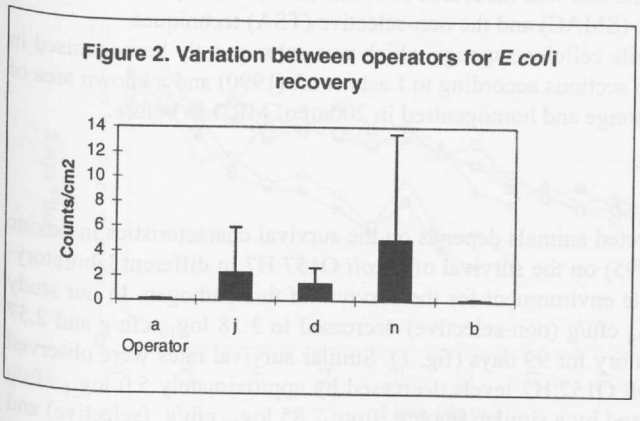
		Petrifilm			MPN		
		Rump	Flank	Brisket	Rump	Flank	Brisket
Swab	mean	0.91	1.74	0.03	1.02	0.56	0.04
	sem	1.39	2.45	0.02	1.11	0.79	0.03
Excision	mean	4.32	4.21	0.26	9.64	5.46	0.39
	sem	2.80	4.16	0.20	6.06	4.85	0.20

Table 2. Comparison of estimation of *E. coli* counts by Petrifilm and MPN

4. Investigation of operator variation in carcass sampling.

Three operators (J,N,D) were experienced laboratory technicians who have had extensive experience in sponge sampling of carcasses. The other two (A,B) were QA officers at the abattoir and had some experience in sampling. See Figure 2 for the men recovery rates per operator. The data were not distributed normally and were analysed by One Way Analysis of Variance on Ranks. There were statistically significant differences in recovery rates for operators ($p<0.0001$). All samples were taken on the same days from the same lot of carcasses and processed the same way. The skilled laboratory operators had better recovery rates than the plant QA officers, who were not assisted or re-trained in any way. The implications for industry are that operators must be adequately trained to take the samples, with on-going audits of skills. Operator variation may cause false low results which may mask an on-going problem on plant.





Conclusions

1. The elimination of the rump from sampling will result in reduced numbers of carcasses falling in the "warning" or "fail" limits of the MegaRegs plan.
2. Sponge sampling recovers less bacteria than excision.
3. Petrifilm method is similar to the MPN method for the enumeration of *E. coli* from sponge samples.
4. There can be considerable variation in recovery of *E. coli* from carcasses by the sponge method from operator to operator.

References:

USDA FSIS Pathogen Reduction Program
Australian Standard 1766.1.3 (1991)
Australian Standard 1766.2.3 (1992)

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