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Prepared by: Trenton Seager[1] Mark L. Tamplin[1*], Jacinta Simmons[1] and John Sumner[2]
[1] Food Safety Centre, Tasmanian Institute of Agricultural Research, University of Tasmania, Meat and Livestock Australia

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Recovery efficiency of total viable counts from beef carcases using the surface sponge sampling method

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Contents

1 Introduction

The safety and quality of beef products are commonly assessed by measurement of Total Viable Counts (TVC) using a surface sponge sampling method (Anonymous, 2002). This test normally involves a single sponging of one to three 100 cm^2 areas on the carcase surface by abattoir operators and inspectors. Each sampler potentially introduces variation based on individual sampling methods. As such, interpretation of carcase TVC levels depends on understanding the inherent uncertainty due to the sampling procedure, which could lead to improved methods with lower test variability.

A comparison of data derived from the Baseline studies and the ESAM database demonstrate that there is an unexplained difference in Total Viable Counts (TVC). To some extent this gap is explained by differences in incubation temperature that we quantified in the 2006-07 MLA Internship study (Simmons et al., 2008). However, additional factors, such as the sampling operator, may influence these results; some operators may rub the carcase surface lightly while others may abrade the meat surface more vigorously, thus removing a larger proportion of the TVC microflora. Sponge sampling so pervades modern meat microbiology that measuring the operator effect is an appropriate project to determine inherent variation in carcase sampling.

This project develops knowledge of operator effect on the recovery of TVC from meat carcase surfaces. The experimental design quantifies the percent of the TVC recovered from the carcase surface based on the force used by the operator to sponge the surface. In addition, measurements of recovery efficiency include the percent TVC removed as a function of multiple sponging events, as well as the age of the carcase ('cold' versus 'hot' sampling). The products of this project will reduce the uncertainty of TVC measurements among different databases and provide a more accurate interpretation of factors that influence the microbiological quality of meat products.

2 Materials and methods

2.1 Experimental design

The experimental designed consisted of three primary objectives:
1. Define the experimental parameters that influence TVC recovery.
2. Determine TVC recovery of beef.
3. Define operator effect on TVC recovery in field trials.

Phase 1 was laboratory-based and involved determining the effect of sequential sponging, force of sponging, efficacy of surfactant in recovery solution and duration of stomaching. Phase 2 applied the sequential sponge test to field conditions while Phase 3 evaluated the differences in TVC recovery for different samplers at five abattoirs.

2.2 Optimisation of experimental parameters

2.2.1 Effect of stomaching duration.

An ethanol-sanitized knife was used to excise a 10 cm by 10 cm by 0.5-1.0 cm section of exterior carcase tissue. Next, 20 ml of 0.1% bacteriological peptone (Oxoid, Basingstoke, UK) were added to stomacher bags before stomaching (Colworth stomacher 400, A.J. Seward & Co. Ltd, London, UK) for 30, 60, 120 and 240 sec. At each time interval, two 1-ml aliquots were removed

and spread on APC Petrifilm (3M, Sydney, Australia) in duplicate, with another 1 ml removed to produce 10- and 100-fold dilutions that were also plated in duplicate on Petrifilm. Petrifilm were incubated at 25ºC for 72 h, colonies counted and data expressed as colony-forming units (CFU) per sponge and per 100 cm^2.

2.2.2 Efficacy of surfactant in recovery solution.

An ethanol sanitized knife was used to excise a 10 cm by 10 cm by 0.5-1.0 cm section of exterior carcase tissue. Each 100 cm^2 section of excised tissue was quartered to produce pieces 5 cm by 5 cm (25 cm^2). Next, 10 ml of 0.1% bacteriological peptone containing 0%, 0.5% (v/v), 1% (v/v) and 2% (v/v) Tween 80 (Merck Pty Ltd, Victoria, Australia) were added to stomacher bags and stomached for 60 sec. Neat and diluted aliquots were spread on APC Petrifilm in duplicate, incubated at 25ºC for 72 h, colonies counted and data expressed as CFU per sponge and per 100 cm^2.

2.3 Recovery of TVC with sequential sampling of beef carcase

2.3.1 Laboratory sampling.

Two separate portions of beef flank (3-d old) were purchased from a local butcher, transported to the laboratory within 45 min and refrigerated at 4ºC. Using an ethanol sanitized knife, 10 cm by 10 cm (100 cm^2) areas were marked on the exterior surface. Sponge swabs were hydrated with 25 ml of Butterfield's phosphate buffer and used to sample individual surface sites by making 10 lateral, reciprocal passes over the site followed by another 10 passes in a 90º direction to the first pass. This was repeated for a total of five individual sponges. Each sponge was placed in a Whirl-Pak bag and two 1 ml aliquots of Butterfield's solution removed and spread on APC Petrifilm in duplicate. Another 1 ml aliquot of expressed sponge was removed from the bag to produce 10-, 100- and 1000-fold dilutions which were spread on APC Petrifilm in duplicate. All Petrifilm were incubated at 25ºC for 72 h, colonies counted and data expressed as CFU per sponge and per 100 cm^2.

2.3.2 Field sampling.

Two 1-d old chilled carcasses were sampled at flank, brisket and rump sites at a single abattoir, following the procedure described in section 2.3.1. After sponge swabbing, all six sites were excised and transferred to stomacher bags. Sponges and tissue were transferred to the laboratory and processed for TVC as described previously. The excised samples were stomached for 2 min in 1% Tween 80 solution, based on results in sections 2.2.1 and 2.2.3. Neat and diluted aliquots were spread on APC Petrifilm in duplicate, incubated at 25ºC for 72 h, colonies counted and data expressed as CFU per sponge and per 100 cm^2.

In a separate experiment, a single hot-boned carcass was sampled at a different abattoir. All sampling and testing methods were as described above.

2.4 Operator effect on recovery

Samples were taken at five different abattoirs by 10 experienced samplers (3 inspectors, 5 operators, 2 researchers), with one carcass sampled per person. A single sponge was used to swab 100 cm^2 areas from flank, brisket and rump sites. The carcase surface tissue was excised and placed in a stomacher bag. All samples were stored in a cool box used to transport samples to the laboratory. Sample TVC were determined as previously described in section 2.3. In

addition, individual sampler techniques were recorded, including description of number of swab strokes, relative pressure rating (scale of 1 to 5, 5 highest), direction of swabbing, and location of swabbing area.

In controlled laboratory experiments, the effect of vertical pressure on TVC recovery by sponging was determined. As previously described in 2.3.1, a 10 cm by 10 cm area on the beef flank was marked with an ethanol-sanitized knife. Areas were excised and placed at the centre of a 15 cm by15 cm plastic box lid using a flame sterilized forceps. Two metal clips were fastened to the edge of the meat and the plastic box to hold the excised sample in place, while double-sided adhesive foam tape was placed on the weighing surface of an electronic balance (Sartorius TE1502S, Edgewood, NY, USA). The plastic lid that held the sample was set on the double-sided tape and the balance tared. Sponge bags were hydrated with 25 ml of Butterfield's phosphate buffer (bioMérieux, Hazelwood, Mo., USA). Pressure was applied to the sponge swab (Whirl-Pak Speci-Sponge, Nasco, Fort Atkinson, Wis., USA) equivalent to 290-340 g measured by the balance, and the surface sponged with the same 10-pass reciprocal, perpendicular procedure described above. When sponging was completed, the document clips were removed and the excised sample transferred to a stomacher bag with flame sterilized forceps. This process was repeated for two additional samples using the same force. The same method was used at a force of 600-700g. Neat and diluted aliquots were spread on Petrifilm in duplicate, incubated at 25ºC for 48 h, colonies counted and data expressed as CFU per sponge and per 100 cm^2.

3 Results and discussion

3.1 Optimisation of experimental parameters

Carcase TVC was determined following the Guidelines which accompany the Australian Standard for production and transportation of meat and meat products for human consumption (Anonymous, 2002). The Standard method was evaluated for determining TVC on the excised carcase tissue. Results showed that a stomaching time of 2 min and 1% Tween enhanced recovery of TVC.

3.2 Effect of sequential sponging on recoverable TVC

Two separate portions of beef flank were analysed in the laboratory to determine the level of TVC recovered by five sequential spongings of a single 100 cm^2 area. Two 100 cm^2 sites were tested per flank. Overall, there was a gradual reduction in recoverable TVC from the 1^{st} to 5^{th} sponging (ave=-0.43 log CFU, sd=0.56, min=-0.03 log CFU, max=- 1.25 log CFU) (Fig. 1). An average of 66% of TVC was recovered after five spongings (total TVC from spongings plus excised tissue divided by total TVC from sponging) (sd=0.10, min=54%, max=78%) (Fig. 2).

Field tests of 100 cm^2 areas on rump, brisket and flank for two carcases also showed a -1.09 log CFU average reduction between the 1^{st} and 5^{th} sponging (sd=0.56, min=-0.42 log CFU, max=-1.7 log CFU). An average of 70% of TVC was recovered after five spongings (sd=0.30, min=11%, max=97%).

Analysis of total TVC recovered, from both laboratory and field data, showed an average of 32 (sd=23%), 48 (sd=24%), 55 (sd=24%), 62 (sd=23%) and 68% (sd=23%) of TVC were recovered with the 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} spongings, respectively. For the 10 carcase sites tested, the five spongings removed an average of 68% (sd=23%) of the total recoverable TVC, within a range of 11 to 97%.

Rump, brisket and flank on a single hot boned carcase was similarly tested and showed than an average of 52% of TVC was recovered after five spongings (sd=0.18, min=35%, max=72%).

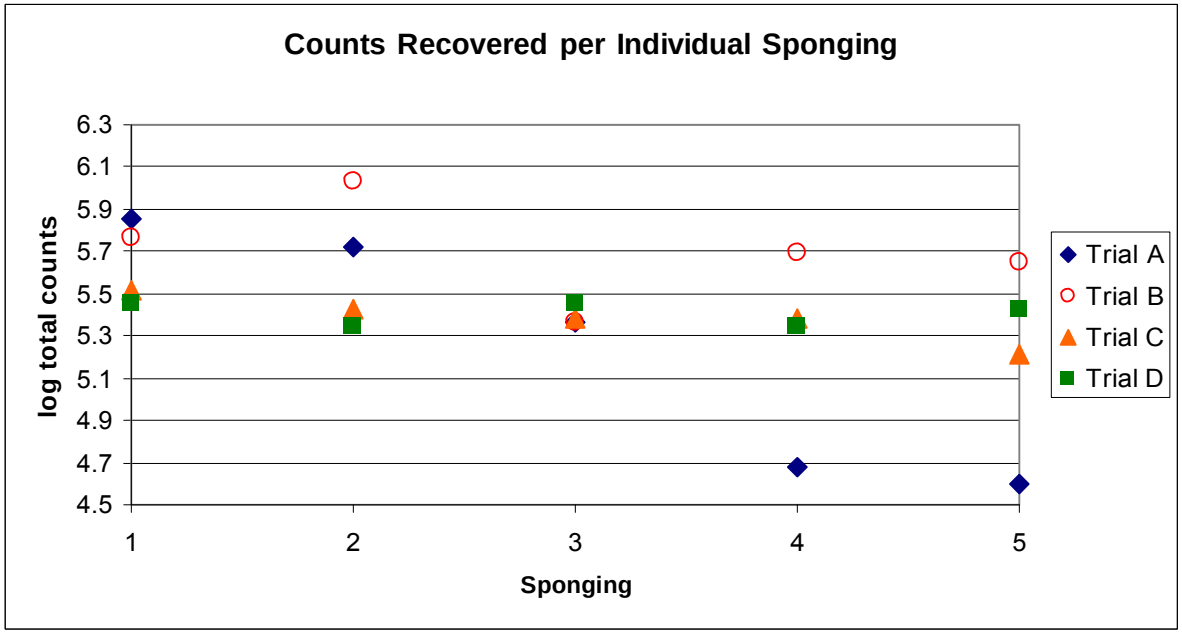

Fig. 1. TVC recovered with five sequential spongings of the same 100cm2 carcase surface area.

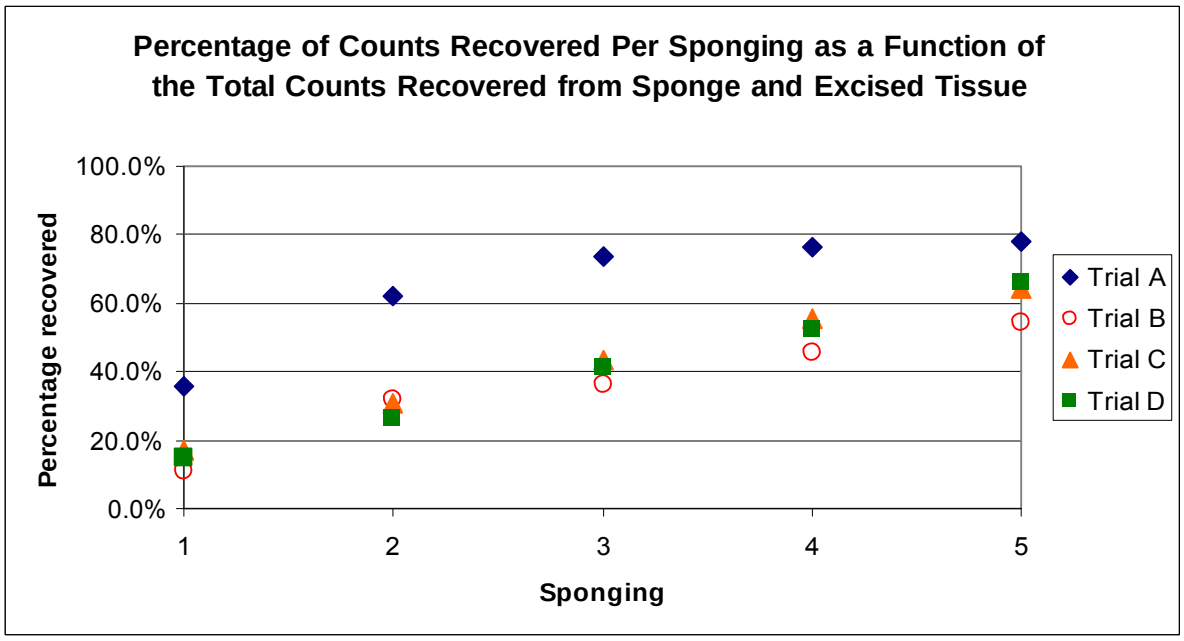

Fig. 2. Percentage of total TVC recovered with five sequential spongings of the same 100cm^2 carcase surface area.

3.3 Operator effect

The Australian Standard procedure for swabbing carcase surfaces is 10 reciprocal strokes in perpendicular directions, using relatively hard pressure (rating of 4-5, on a 1 to 5 scale). For the 10 different samplers at the five different abattoirs, sponging techniques varied considerably. Relative pressure ranged from 2 to 5 with an average of 3.4; six samplers exceeded the standard number of swabbing strokes; two samples swabbed an area larger that 100 cm^2; five samplers used unidirectional strokes versus reciprocal. Also, two samplers doubled-over the sponge, thus reducing the sponge collection area. Yet, with such variation in swabbing techniques, no relationship was observed between swabbing force and percentage TVC recovered.

Separate laboratory-controlled experiments demonstrated that twice the downward force on the sponge recovered more TVC (Fig. 3). However this relationship was likely over-shadowed in the field when combined with other sampling influences, such as friction, number of swabbing strokes, swabbing direction and variation in attachment properties of surface bacteria.

Analysis of the percent TVC recovered from each of the three carcase surface sampling sites showed that the precent recovery ranged from ~10 to ~60% (Fig. 4). There was a broader distribution for rump than for flank or brisket.

The distribution of percent recovery for combined carcase sampling sites showed a peak between 30 and 50% recovery, with an average of 37% (sd=23) (Fig. 4). This indicates that the standard method does not recovery approximately 63% of the detectable TVC count.

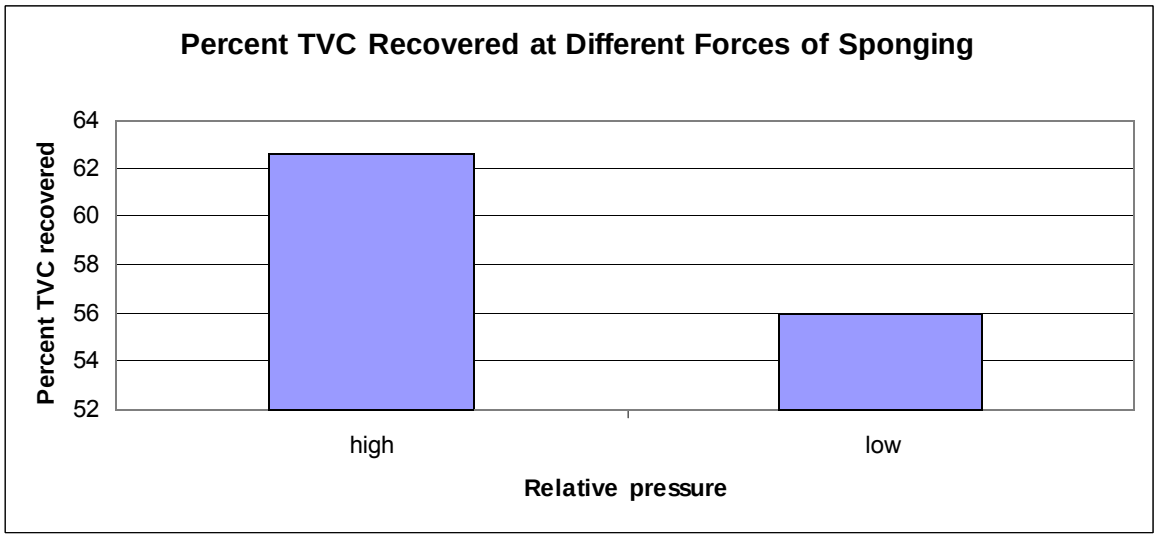

Fig. 3. Percentage of total TVC recovered using low (290-340 g) and high (600-700g) downward pressure on 100cm^2 flank surface.

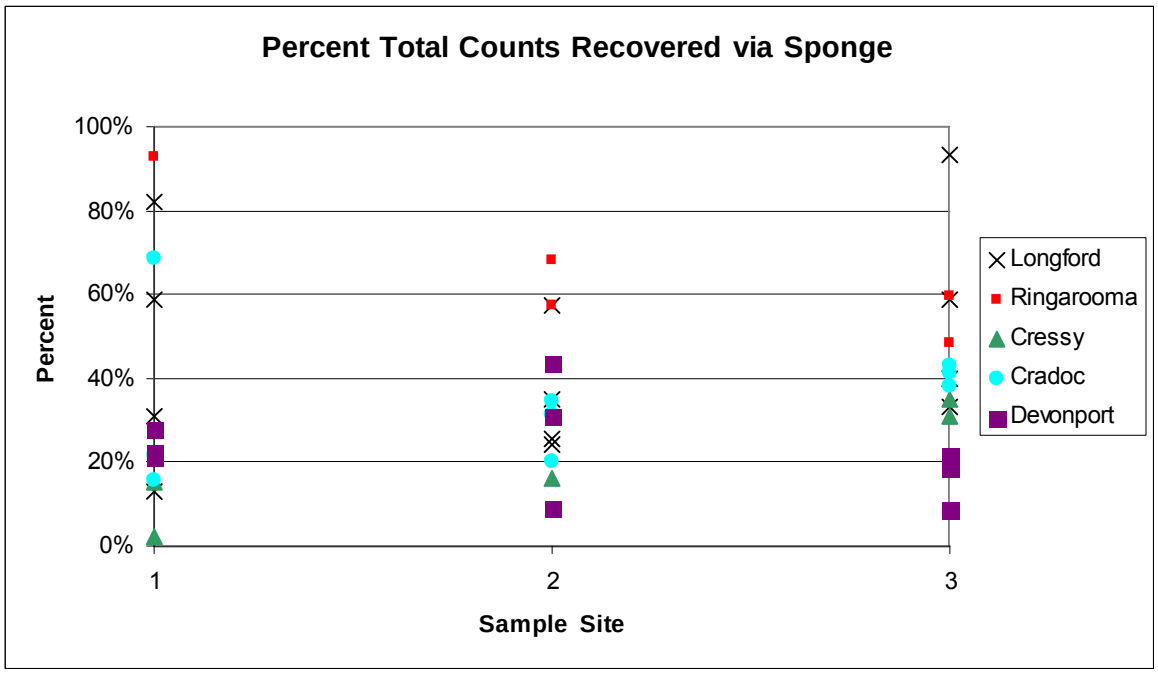

Fig. 4. Percentage of total TVC recovered from beef carcase surface sites. Sample site: 1=rump, 2=brisket, 3=flank.

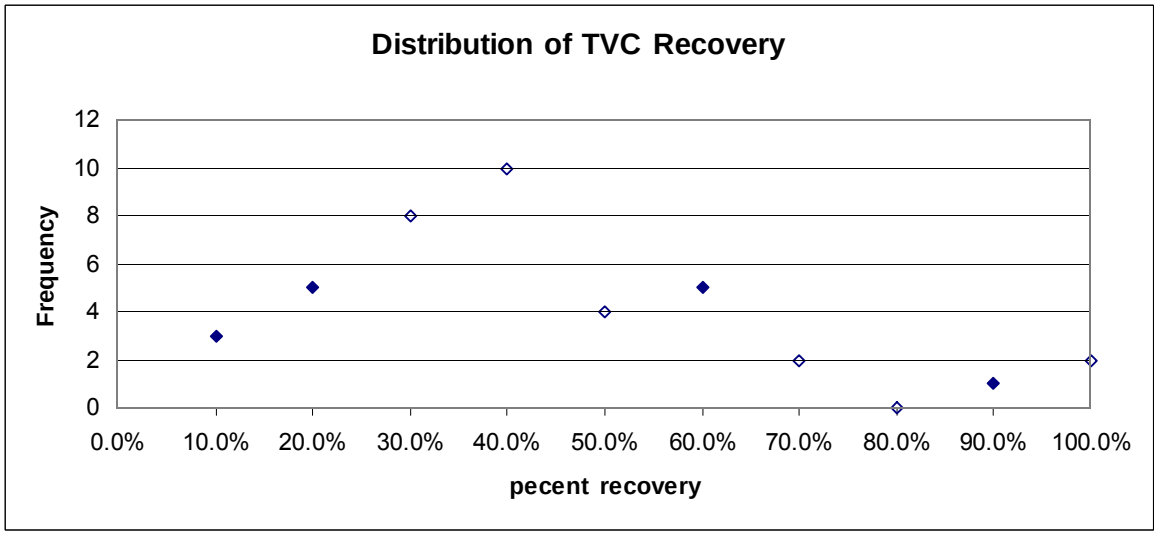

Fig. 5. Distribution of the percentage TVC recovered from all carcase sampling sites.

4 Reference

Anonymous. 2002. Australian Standard for production and transportation of meat and meat products for human consumption, AS 4696:2002.

Simmons, J., Tamplin, M.L., Jenson, I., and J. Sumner. 2008. The effect of incubation temperature on aerobic plate counts of beef and sheep carcasses. J Food Protect. 71:373-375.