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A comparison of swab and maceration methods for bacterial sampling of pig carcasses

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SUMMARY

A swabbing technique was compared with an excision and maceration technique for bacteriological sampling of pig carcass skin surfaces. Total viable counts at 37 °C obtained by swabbing were 46% of those obtained by maceration. At 21 °C, swabbing gave total viable counts which were 54% of the counts obtained from excision samples. *Escherichia coli* counts showed wide variation with both sampling methods. Neither method was more efficient than the other in recovering *E. coli*, although excision sampling gave generally higher counts. Both methods were equally effective at recovering salmonellae from carcass surfaces. There was no significant difference between the methods in recovering particular *Salmonella* serotypes.

INTRODUCTION

The methods used to collect microbial samples from carcasses should be simple, non-destructive, reproducible and economical (Scholefield, Menon & Lam, 1981). Excision of a section of the surface to be sampled gives the most reliable picture of microbial contamination on meat surfaces (Ingram & Roberts, 1976), and is used as a standard against which all other sampling techniques are evaluated. This technique is not, however, practical in a commercial environment when such sampling may produce unsightly mutilation of carcasses. Other sampling methods which involve scraping, swabbing, adhesion, as in the agar contact method, or washing off are only partially effective at recovering microbial populations from carcass surfaces (Ingram & Roberts, 1976). For example, Ojala (1964) showed that swabbing was only 16% effective, compared to excision, in recovering bacteria from meat surfaces. In spite of this lack of sensitivity, swabbing is still the most universally used method for the estimation of bacterial populations on meat and other surfaces because it is simple and non-destructive. While there is an extensive literature on the effectiveness of various sampling methods for recovery of bacteria from red meat surfaces, there is only limited information available on their use for microbial sampling of pig carcasses. The effectiveness of surface sampling techniques depends on the surface to which they are applied (Nortje *et al.* 1982) and it does not follow that results from red meat surfaces will be the same as results from pig skin. The aim of this work was to determine whether swabbing could

be used as a practical, non-destructive microbial sampling technique for examining microbial contamination of pig carcasses during the slaughtering and dressing process.

MATERIALS AND METHODS

Pieces of jowl skin, approximately 20 cm by 20 cm, were collected from pig carcasses at two abattoirs. Abattoir A used tank scalding, while abbattoir B used a steam chamber for scalding pig carcasses. Both abattoirs used beater-dehairers of similar design, but abattoir B used two machines in tandem while the other abattoir had a single machine. Both abattoirs carried out manual scraping and singeing of carcasses to remove residual hair from carcasses. At each visit to the abattoirs jowl skin pieces were collected from ten successive carcasses on the slaughter line, prior to the final wash and trim section. Skin pieces were transported on ice to the laboratory in individual sterilized containers.

Sampling of skin surface

On arrival at the laboratory the outer surface of each skin piece was rubbed on itself to give an even distribution of organisms over the whole surface, following the method of Anderson *et al.* (1980). From each skin piece a 20 cm^2 sample was cut out using a sterilized stainless steel punch. In addition, two 20 cm^2 areas were swabbed with sterile alginate swabs using the wet and dry swab technique of Kitchell, Ingram & Hudson (1973). The areas swabbed were marked with a sterile metal template. The swabs were collected into peptone water. The swabs used to sample the 40 cm^2 area and the skin sample were individually stomached in a Colworth Stomacher (Seward, England) with 20 ml of 0·1 % peptone water as the suspending medium.

Bacteriology

Total viable counts and *Escherichia coli* counts were performed on the samples. The samples were also examined for the presence of salmonellae.

Total viable counts

Total viable counts (TVC) were determined by the agar droplet technique of Sharpe & Kilsby (1971) using a Colworth droplette machine (Seward, England). One decimal and one centimal dilution of the homogenate were prepared in 9·0 ml and 9·9 ml volumes respectively, of Plate Count Agar (Oxoid) held at 45 °C. The Colworth diluter/dispenser machine (Seward, England) was used to place five 0·1 ml droplets from each of the decimal and centimal dilutions on to a standard Petri dish. Two sets of plates were prepared from each sample, one sample was incubated at 37 °C for one day and the other at 21 °C for two days. The plates were incubated in a humid environment to prevent the droplets from drying out. Colonies were enumerated using the projection viewer on the droplette machine. Colonies were only counted when there were more than 20 per droplet and the mean count for the five droplets was estimated.

E. coli counts

E. coli were counted by the direct plating technique of Anderson & Baird-Parker (1975). A 4 h resuscitation step was included (Holbrook, Anderson & Baird-Parker,

1980) to recover sublethally damaged *E. coli*. Cellulose acetate membranes, 85 mm diameter, 450 nm pore size (Oxoid Ltd) were placed on pre-dried surfaces of Minerals Modified Glutamate Agar (MMGA) (Oxoid). The membranes were gently flattened on to the surface with a sterile glass spreader, and 0·5 ml of homogenate was evenly spread on to the membrane and allowed to adsorb for 30 min. Plates were incubated at 37 °C for 4 h. Membranes were then transferred aseptically from the MMGA to pre-dried Tryptone Bile Agar plates (TBA) (Oxoid) and incubated for 20 h at 44 °C. To detect indole-positive colonies the membrane was removed from the TBA and immersed into 2 ml of indole reagent (Vracko & Sherris, 1963) in a Petri dish lid for 5 min. After removal from the reagent the membranes were then dried under a low-pressure ultraviolet lamp. The pink indole-positive colonies were enumerated as *E. coli*.

Salmonella isolation

The remainder of the homogenate was added to an equal volume of double-strength buffered peptone water with 2% Tergitol (BDH) and incubated at 37 °C for 18 h. One ml of the pre-enrichment sample was transferred into Mannitol Selenite Broth (MS) (Oxoid) with 0·01% L-cystine, and a further 1 ml was transferred to Tetrathionate Broth (Tet) (Oxoid) with 1:100000 brilliant green. In addition, 100 μl of the pre-enrichment sample was inoculated into 10 ml of Rappaport–Vassiliadis broth (RV) (Vassiliadis *et al.* 1981). The MS broth was incubated at 42 °C, the Tet broth at 37 °C and the RV broth at 43 °C. After 18–24 h incubation each of the selective enrichment broths was plated on to Brilliant Green Sulpha Agar (BGS) (Gibco), Xylose Lysine Deoxycholate Agar (XLD) (Gibco) and Bismuth Sulphite Agar (BS) (Gibco) plates. The BGS and XLD plates were incubated for 24 h at 37 °C and BS plates for 48 h at 37 °C. After incubation plates were examined and up to two suspect salmonella colonies were picked from each plate and submitted to biochemical and serological identification procedures. The identity of all salmonella isolates were confirmed at the Microbiology Diagnostic Unit, University of Melbourne.

Data analysis

TVC and *E. coli* counts were converted to logarithmic (to the base 10) values/cm^2 for statistical analysis including linear regression (Sokhal & Rohlf, 1969).

RESULTS

Thirty jowl skin pieces were collected from pigs at abattoir A on 3 visits and 50 jowl skin pieces from abattoir B over 5 visits. The results of the TVC and *E. coli* counts are presented in Table 1.

Total viable counts at 37 °C

There was no significant difference ($P < 0·05$) in the average bacterial levels on jowls collected from the two abattoirs when measured by the swab or excision method. There were, however, significant differences between the counts measured by the swab method compared with the excision method, with the latter being significantly ($P < 0·05$) higher. Aggregated values from the two abattoirs showed

Table 1. *Comparison of excision and swab technique for estimating total viable counts and* E. coli *numbers on pig skin*

			37 °C Total viable counts			21 °C Total viable counts			*E. coli* counts		
Works	Technique	No. of samples	Mean	S.E.	Correlation with excision technique (r^2)	Mean	S.E.	Correlations with excision technique (r^2)	Mean	S.E.	Correlation with excision technique (r^2)
A	Excision	30	4·036*	0·072		3·969	0·058		1·784	0·169	
	Swab	30	3·712	0·063	0·34†	3·783	0·057	0·09	1·535	0·156	0·36†
B	Excision	50	4·074	0·050		4·016	0·052		1·732	0·127	
	Swab	50	3·735	0·057	0·31†	3·723	0·050	0·41†	1·514	0·124‡	0·72†
Total	Excision	80	4·060	0·041		3·999	0·039		1·752	0·101	
	Swab	80	3·727	0·043	0·32†	3·746	0·038	0·28†	1·522	0·096‡	0·56†

* Mean $\log_{10}$ count/cm² of skin sampled.

† $P < 0·05$.

‡ Two swab samples were overgrown and *E. coli* counts could not be determined. The mean counts are calculated from 48 samples. The total number of swab samples examined for *E. coli* was 78.

Table 2. *Relationship of total viable counts and* E. coli *numbers to the presence of* Salmonella *spp.*

			37 °C Total viable counts		21 °C Total viable counts		*E. coli* counts	
Sampling technique	Salmonella detected	No.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Swab	+	31	3·89*	0·063	3·90	0·049	1·85	0·115
	−	49	3·62	0·052	3·65	0·048	1·33	0·131
Excision	+	27	4·14	0·076	4·11	0·069	1·87	0·118
	−	53	4·02	0·048	3·94	0·046	1·69	0·140

* Mean $\log_{10}$ count/cm² of skin sampled.

a similar pattern. The geometric mean of the counts on the 80 jowls determined by the swab method was 5333/cm², whilst for the excision method the geometric mean was 11480/cm². The average count obtained by the swab method was 46% of the average count obtained by the excision method.

There was a significant correlation between the counts obtained by the swab method and the excision method ($r^2 = 0{\cdot}32$; $P < 0{\cdot}01$). The correlation between the two methods is shown in Fig. 1*a* together with the regression equation.

Total viable count at 21 °C

There was no significant difference in the average counts obtained from jowls from the two abattoirs when measured by either the swab or excision method. At abattoir B, but not at abattoir A, the counts obtained by the excision method were significantly higher than those obtained by the swab method. Aggregation of the counts from both abattoirs showed that overall the excision method gave a significantly higher count than the swab method. The geometric mean count was 9977/cm² for the excision method, compared to 5572/cm² for the swab method. The swab method gave 56% of the average count obtained by the excision method.

There was a significant correlation between the counts obtained by the swab method and those obtained by the excision method ($r^2 = 0{\cdot}28$, $P < 0{\cdot}01$). The correlation between the counts obtained by the two methods is shown in Fig. 1*b* together with the regression equation.

E. coli *counts*

There was no significant differences in the average counts obtained from jowls collected from the two abattoirs by either the swab or excision method. The geometric mean count was 56/cm² for the excision technique and 33/cm² for the swab technique.

There was a significant correlation between the counts obtained by the swab method and those obtained by the excision method ($r^2 = 0{\cdot}56$, $P < 0{\cdot}01$). The correlation between the two methods is shown in Fig. 1*c*, together with the regression equation.

Salmonella isolations

Salmonella were isolated from 35 of the 80 jowls, an isolation rate of 43·75%. The swab technique failed to detect salmonellas on four occasions when salmonellas were isolated from a jowl. The excision technique failed to detect salmonellas on nine occasions. McNemar's test for correlated proportions shows that the difference in the proportion of salmonellas isolated by the two methods is not significant ($\chi^2 = 1{\cdot}02$).

Forty-six strains of salmonellas representing six serotypes were isolated from the 35 jowls. The frequency of serotype isolation was *S. give* (17/46, 37%), *S. anatum* (13/46, 28%), *S. derby* (6/46, 13%), *S. typhimurium* phage type 126 (6/64, 13%), *S. ohio* (3/46, 7%) and *S. typhimurium* phage type 4 (1/46, 2%). There was no significant difference in the isolation rate of particular serotypes by the two methods.

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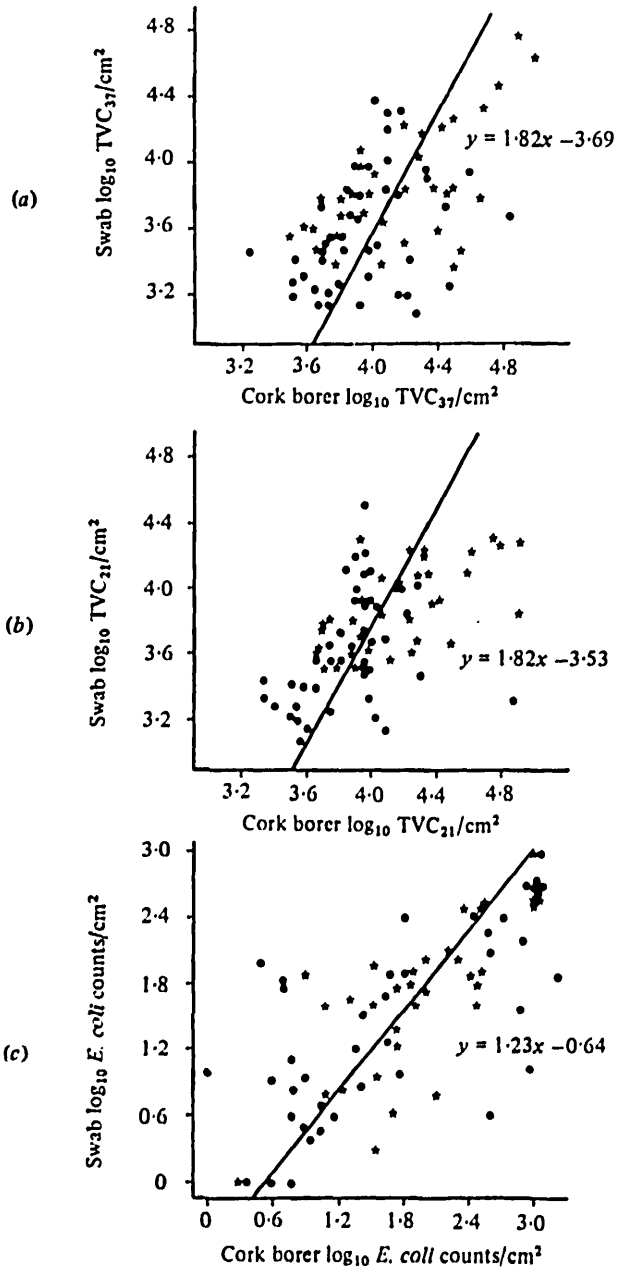

Fig. 1. Correlation between excision and swab techniques for estimating total viable counts and *E. coli* numbers on pig skin. (*a*) 37 °C TVC; (*b*) 21 °C TVC; (*c*) *E. coli* counts. ★, Salmonella; ●, non-salmonella.

Bacterial counts from salmonella-contaminated jowls were compared with counts from jowls from which salmonellas were not isolated. Mean counts are shown in Table 2 and the salmonella positives are shown on Fig. 1 *a*, *b* and *c*. The swab technique showed significantly higher total viable counts from salmonella-contaminated jowls at both 37 and 21 °C. The excision technique showed a significantly higher total viable count on salmonella-contaminated jowls at 21 but

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not at 37 °C. Irrespective of the sampling method used, the *E. coli* counts obtained from salmonella-contaminated and uncontaminated jowls were not significantly different.

DISCUSSION

An excision technique using 20 cm² of skin was compared with a swab sample from 40 cm². The smaller area for the excision technique, representing a 5 cm diameter patch, was thought to be the maximum practical size for sampling. However, even a sample of this size could disfigure a carcass, and most workers have used only a few square centimetres for excision sampling (Ingram & Roberts, 1976). As bacteria are randomly distributed on a meat surface, the likely variation in sampling reduces as the sampling area increases (Ingram & Roberts, 1976). The area to be swabbed was selected to ensure the largest surface possible was sampled consistent with practical template sizes. On carcasses the difference in sampling area might have influenced the results obtained due to variation in distribution of bacteria over the surface. In this investigation an attempt was made to distribute bacteria evenly over the surface sampled in order to reduce the sampling variance following the method of Anderson *et al.* (1980). For this reason it is believed that the differences in the areas sampled by the two methods have not influenced the results.

In this investigation the TVC at 37 °C obtained by swabbing was on average 46% of that obtained by excision and maceration. At 21 °C swabbing gave TVCs which were, on average, 54% of the counts obtained by excision and maceration. This is similar to the result reported by Ingram & Roberts (1976), who obtained swab TVCs at 37 °C from pig carcasses which were 44% of those obtained by excision. Scholefield, Menon & Lam (1981) showed that only 22% of the 30 °C TVC bacteria obtained by excision were recovered by swabbing. However, in this case the area sampled was 2·5 cm², compared with the 20 cm² and 40 cm² sampled in this experiment and 50 cm² sampled by Ingram & Roberts (1976). The latter have noted that the size of the area sampled affects the efficiency of recovery of microorganisms from a surface.

These results from sampling of pig carcasses compare favourably with those obtained in red meat sampling, where swab sampling often yields a TVC of 16% or less of that obtained by excision (Ojala, 1964; Ingram & Roberts, 1976). The result may be due to the nature of the surface being sampled. The scalded skin of the pig carcass could provide a surface which allows swabbing to remove more bacteria than from a red meat surface. This difference may be related to the fat composition of the surface being sampled (Ingram & Roberts, 1976).

The results obtained suggest that pig carcasses appear to have a relatively uniform level of microbial contamination, as evidenced by the standard errors of the mean counts. Total viable counts obtained by swabbing correlated well with those obtained by the excision technique (Fig. 1*a*,*b*). The regression equations could be used to predict the excision count from a swab count. Swabbing detected *E. coli* counts that were 58% of those obtained by excision. However, because of the wide variation of the counts obtained by both sampling methods the mean *E. coli* counts were not significantly different. Both methods were equally variable, suggesting that one method was not superior to the other in reducing the

variability of the counts. The correlation between the *E. coli* counts obtained by swabbing and those by excision was good (Fig. 1*c*), suggesting that the regression equation could be used to predict the excision count from a swab count.

Both sampling methods were equally efficient at detecting salmonellas although, in spite of efforts to distribute organisms evenly over the whole of the jowl sampled, both methods failed to recover salmonellas on several occasions. This suggests that these organisms may be attached to the skin surface and less easily dislodged by maceration or swabbing. Alternatively, the organisms may have been present in small numbers and been irregularly distributed when the surfaces were rubbed together.

Total viable count and *E. coli* count values could not be used to predict the presence of salmonellas (Fig. 1). However, TVC and *E. coli* counts detected by the swab method were significantly higher when salmonellas were recovered from the jowls sampled.

The conclusion which can be drawn from this investigation is that swabbing can be used as an effective, simple, non-destructive method for estimating bacterial contamination of, and recovery of salmonellas from, pig carcasses.

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