COMPARISON OF TECHNIQUES TO EVALUATE THE BACTERIOLOGICAL QUALITY OF PIG CARCASS SURFACES.

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Background: Animal carcasses can become contaminated with fecal bacteria during various slaughter operations. In beef, this contamination comes from the hide when the animal is skinned and/or intestinal contents due to leakage/breakage. In pork, since the carcasses are not usually skinned, the contamination comes from what remains on the surface after scalding, dehairing, washing, and polishing and/or intestine either because of leakage or breakage.

Different techniques have been devised to determine the bacterial flora of animal carcass surfaces. These include: a) contact plates (Miller et al, 1994); b) various tape procedures (Fung et al, 1980); c) swabbing methods (Dorsa et al, 1996; Carpenter et al, 1973; Gill et al, 1996); and d) excision methods (Dorsa et al, 1996). Each has its advantages and disadvantages, and thus each could indicate a different level of contamination. This study began with an inquiry from the USDA's Food Safety and Inspection Service (FSIS) for a means to compare the results of a swabbing method to that of the standard excision method for sampling pig carcasses. Since bacteriological sampling of animal carcasses is expected to become a standard practice by establisments and inspection personnel, FSIS was seeking to validate a useful and less labor- and equipment-intensive swab method.

Objectives: The objectives of this study were to compare: a) recovery of the total aerobic count using swab vs. excision methods; b) the use of a double vs. a single swab procedure for recovering the total aerobic counts; c) recovery from different sampling site on the carcass (rump [ham] vs. belly [brisket] vs. flank [shoulder]) for swab vs. excision methods; d) recovery immediately post slaughter vs. recovery from chilled carcasses and; e) methods to quantitatively recover *Salmonella* inoculated at different levels onto fresh pig skin.

METHODS

General. To allow comparison of our data with the FSIS national baseline survey data, a standard 60 cm^2 (7.75 x 7.75 cm) template was employed in all experiments in this study. The template was sterilized by autoclaving at 121°C for 20 min initially and then by dipping in 70% ethanol during use between samples. The samples were processed within 15-30 minutes in a laboratory located adjacent to the slaughter floor. The carcass sites were sampled either immediately post slaughter (after the final rinse) or post chilling (24 hrs at 2±1°C). For the swab *vs.* excision comparison, the swab sites were located from right or left half of the carcass and the excision samples were taken from the matched half of the same carcass.

Excision. An approximately 100 cm^2 area (about 2-3 cm deep) of skin and underlying tissue was removed from the three sites (rump [ham], belly [brisket] and flank [shoulder]) and transported to the laboratory in a sterile covered tray. In the laboratory, a 60 cm^2 area of skin, as outlined by the template, was aseptically removed from the sample using sterile scalpel and forceps and placed in a sterile Stomacher bag. Twenty-five ml of buffered peptone water (BPW; Difco) were added and the contents massaged for two minutes using a Stomacher Laboratory mixer. One ml aliquots or 1:10 dilution made in 0.1% peptone water were plated in duplicate on to total plate Count Petri film™ and the plates were hand counted after 24 hrs at 37°C.

Swab Method. A 60 cm^2 area was swabbed using a pre-moistened (10 ml of BPW) Nasco sponge . After swabbing, the sponge was returned to a sterile Stomacher bag and brought to the laboratory. An additional 15 ml of BPW were added and the entire contents mixed for two minutes in a Stomacher Laboratory mixer. One ml aliquots or 1:10 dilution made in peptone water were plated on Petri film as described above.

Double Swab Method. This method consisted of the swab method described above followed by second swabbing of the area with a sterile cotton ball. This cotton ball was placed in the Stomacher bag along with the sponge and contents processed as with the swab method.

Salmonella. A rifampin-resistant mutant of *Salmonella typhimurium* ATCC 14028 was used in these studies. An overnight culture was grown in tryptic soy broth (Difco, 37°C with shaking) and then diluted in sterile fecal diluent (one part fresh pig feces + 9 parts distilled water, autoclaved in a Stomacher filter bag, and the clear fluid passing through the bag membrane. Dilutions of the culture were then placed on skin removed from a freshly slaughtered pig and allowed to remain refrigerated for 24 hrs at 4°C to simulate plant handling practice. After 24 hrs, areas were either swabbed or excised at described above. For the swab sample, the diluent and sponge were divided into two equal portions; one half was frozen for 8 days at -18°C and one was analyzed immediately for *Salmonella* as follows: pre-enrichment in BPW for 24 hrs at 37°C, enrichment in tetrathionate broth (Difco; Tet) for 24 hrs at 42°C and selenite-cystine (Difco;Sc) for 24 hrs at 37°C. The enrichment cultures were then streaked to double modified lysine iron agar; presumptive *Salmonella* colonies (black colonies surrounded by an alkaline reaction) were confirmed by streaking onto tryptic soy agar (Difco) + rifampin.

Data. All data were analyzed using the SAS program.

RESULTS AND DISCUSSION

Our initial evaluation indicated a small but statistically not significant increase ($P > 0.05$)in recovery of aerobic plate count bacteria using the double swab method compared to the single swab method (Table 1). The limited increased recovery did not seem to warrant the addition effort required to further evaluate the double swab technique. It was also observed that chilling of the carcasses significantly reduced the number of bacteria which could be recovered (Table 1). The basis for this reduced recovery is not known, but the bacteria may have become more firmly attached and thus not removed by the swab techniques and/or the low temperature and a_w on the skin surface may have inactivate the bacteria.

While higher recovery was observed pre-chill, standard practice dictates sampling post-chill and all further evaluations were done on post-chill carcasses. Swab using a sponge was compared with excision for 29 carcasses at three carcass sampling sites. As with other comparisons made thus far, there was overall no statistical difference between the recovery of aerobic bacteria by the two methods (Table 2). Both methods appear to be equally satisfactory at recovering bacteria from the surfaces of these pigs. It is not known at this time whether the similar recovery is due to low starting numbers or other factors. Sampling site and method were next compared. These data are present in Table 3. As can be seen in this table, recovery was higher for both rump and flank samples taken by excision compared to the swab method; for brisket samples, however, both methods yielded similar recoveries. The basis of these similarities and differences are not known, but since the rump is near the rectal area, this could account for higher numbers, particularly of bacteria which are not removed by the washing steps used. Higher recoveries by excision from the flank area could represent bacteria both as part of the carcass flora and those washed down from other area of the carcass.

Various factors which contribute to the recovery of *Salmonella* from pig skin were evaluated. Before frozen storage, swab and excision yielded similar recoveries of *Salmonella*, with both enrichment broths functioning equally well. Either broth recovered all levels of *Salmonella* inoculated onto the skin. As expected, the number of *Salmonella* recovered declined after freezing, but both Sc and Tet appeared to give equivalent recovery of this bacterium.

CONCLUSION: When recoveries from the three carcass sites are analyzed together, both swab and excision gave similar pictures of the bacterial quality of a pig carcass (Table 2). However, when the sites are analyzed separately, excision is better for rump samples and slightly better for flank samples (Table 3). Further, either swab or excision appear equally satisfactory for recovery of *Salmonella* (Table 4).

PERTINENT LITERATURE

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Table 1. Effect of sampling time and method on the aerobic plate count of pig carcasses (based on samples from 10 carcasses,belly region).

METHOD	PRE-CHILLED, TOTAL PLATE COUNT	POST-CHILLED, TOTAL PLATE COUNT
SWAB	33.9[a]	8.0[b]
DOUBLE SWAB	42.0[a]	12.5[b]

[ab] Values in column with the same superscript are not significantly differentat the 95% confidence level.

Table 2. Comparison of swab *vs.* excision on the aerobic plate count of pig carcasses (based on 29 carcasses, three site sampled and all three sites averaged together).

METHOD	TOTAL PLATE COUNT
SWAB	84.5[a]
EXCISION	113.4[a]

[a] Values in column with the same superscript are not significantly different at the 95% confidence level

Table 3. Effect of sampling site and method on the aerobic plate count of pig carcasses (based on 29 carcasses, three sites sampled and all three sites averaged together).

	Method	
Site	Swab	Excision
ham (rump)	63.2[a]	115.8[b]
belly (brisket)	115.8[b]	111.2[b]
flank (shoulder)	74.9[c]	113.0[d]

[ab] Values in row with the same superscript are significantly different at the 95% confidence level.
[bb] Values in row with the same superscript are not significantly different at the 95% confidence level.
[cd] Values in row with the same superscript are significantly different at the 90% confidence level.

Table 4. Effect of sampling method (swab *vs.* excision), storage time (fresh *vs.* frozen) and enrichment medium (selenite cystine [Sc] *vs.* tetrathionate [Tet]) on the recovery onto pig skin.

STARTING LEVEL OF *Salmonella* per cm^2*	EXCISION	SWAB	SWAB AFTER 8 DAYS STORAGE AT -18°C	
	similar recovery in Sc and Tet	similar recovery in Sc and Tet	Sc	Tet
0 (control)	0+/5**	0+/5	0+/5	0+/5
1.12	5+/5	5+/5	2+/5	1+/5
1.16×10^1	5+/5	5+/5	4+/5	3+/5
1.12×10^2	5+/5	5+/5	5+/5	5+/5
$1,05 \times 10^3$	5+/5	5+/5	5+/5	5+/5

* Level from plate counts (TSA for 24 hrs at 37°C) of dilutions applied to fresh pig skin.
** Number of 60 cm^2 areas tested positive/number of 60 cm^2 areas sampled.