

Bacterial populations on dressed pig carcasses

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SUMMARY

Samples were collected at two abattoirs from four sites on pig carcasses as they were being placed in a chiller. Bacteriological examination showed that no single sampling site could be used to assess the microbiological status of pig carcass surfaces. Sampling from multiple sites on a carcass may be required to assess the degree of contamination by different bacteria. It is suggested that the hygiene of slaughtering and dressing of pig carcasses at an abattoir cannot be assessed on a single visit and that a number of visits are necessary to establish a hygiene pattern.

INTRODUCTION

The bacterial population on the surface of carcasses, at the end of the slaughter process, may reflect the hygiene practised at various stages in the slaughter and dressing process (Roberts, McFie & Hudson, 1980). Ingram & Roberts (1976) noted that micro-organisms are not uniformly distributed over carcass surfaces and that the heavily contaminated sites may vary from carcass to carcass. There may however be consistent differences, on average, between different parts of a carcass. If heavily contaminated sites are defined it should be possible to reduce the costs of bacteriological testing and to more accurately compare hygiene in different abattoirs. Sampling of such sites could reduce the variability of bacterial counts, allowing fewer animals to be examined to detect differences in hygiene practices. The aim of the investigation reported in this paper was to determine levels of bacterial contamination of pig carcasses, in commercial environments, and to identify the most heavily contaminated sites on pig carcasses. This information was needed to design sampling procedures that identify points in the slaughter chain which influence bacterial contamination of pig carcasses.

MATERIALS AND METHODS

The survey was carried out between October and December at two abattoirs. Abattoir A used a steam chamber for scalding and tandem beaters for dehairing while Abattoir B used a tank scalding process and a single-beater dehairer. Both abattoirs lightly singed carcasses by means of hand-held gas torches. Each abattoir was visited on five occasions when ten pig carcasses were sampled at the end of the slaughterline as they were placed in chiller storage. Carcasses were sampled at the same time of day at each visit, approximately 30 min after slaughtering

commenced. Samples were taken at random intervals to reduce the chances of sampling carcasses which might have been contaminated by occasional break-downs in hygiene on the slaughterline, such as gut spillage. Samples were collected from four sites on each carcass: (1) dorsal to the tail near the anal opening; (2) lateral surface of the hind leg approximately mid-way on a line between the hip and stifle joints; (3) mid-dorsal region; (4) lateral surface of the cheek-jowl. Two 20 cm² areas were sampled at each site, on opposite halves of the carcass, using sterile templates and the wet-dry swab technique of Kitchell, Ingram & Hudson (1973). Swabs from the two areas sampled at each site were combined in 20 ml of 0.1 % peptone water (Oxoid) for transport to the laboratory on ice.

Bacteriology

The swabs used to sample the 40 cm² areas were blended in a Colworth Stomacher (Seward, England) with the 20 ml of peptone water used to transport the swabs to the laboratory. Total viable counts and *Escherichia coli* were performed on the homogenates, which were also examined for the presence of salmonellas.

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 ($\frac{1}{10}$) and one centimal ($\frac{1}{100}$) 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 being incubated at 37 °C for 1 day and the other at 21 °C for 2 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, then the mean count for the five droplets was estimated.

E. coli counts

Escherichia 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) 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 on to 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 broth 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 was confirmed at the Microbiology Diagnostic Unit, University of Melbourne.

Statistics

All counts were converted to logarithms to the base 10 and used in an hierarchical design, nested analysis of variance to examine the effect of sampling day, site and abattoir on bacterial populations. The statistical analysis was performed using the GENSTAT package (Alvey, Galwey & Lane, 1982) with the hierarchy of levels being abattoirs, visits, animals, sampling sites on animals. Differences between means were examined by *t* test (Alvey, Galwey & Lane, 1982). McNemar's test for correlated proportions was used to analyse the rate of salmonella isolation at the four sampling sites.

RESULTS

The mean TVC at 37 °C and 21 °C and the *E. coli* counts at each visit, for all carcass sites, are presented in Table 1. The distribution of the bacterial counts, over the five visits at each abattoir, for all four sites are shown in Table 2.

In general, the TVC incubated at 21 °C gave slightly higher mean counts than those obtained at 37 °C at Abattoir A, and markedly higher counts at Abattoir B.

Total viable count at 37 °C

At Abattoir A animals, visits and sampling site had a significant effect on TVC value (Table 3). There was no significant interaction between visit and sampling site and between animal and sampling site. The visit effects and animal effects were therefore consistent over all sampling sites. When sites were compared, site 3 (mid-dorsal region) TVC were significantly lower than those at site 1 and site 2, but not significantly different from those at site 4 (Table 4).

At Abattoir B animals, visits and sampling site all had significant effects on TVC value (Table 3). There were significant interactions between visit and site, so that

Table 1. Mean and standard errors for bacterial counts (\log_{10}/cm^2) at four sites on pig carcasses

Abat- toir	Visit	Total viable counts at 37 °C				Total viable counts at 21 °C				E. coli count															
		1	2	3	4	1	2	3	4	1	2	3	4												
		X	S.E.	X	S.E.	X	S.E.	X	S.E.	X	S.E.	X	S.E.	X	S.E.	X	S.E.								
A	1*	3.46	0.09	3.37	0.08	3.32	0.10	3.18	0.10	3.25	0.08	3.13	0.08	3.11	0.12	3.01	0.08	0.97	0.22	1.01	0.28	0.55	0.15	0.58	0.13
	2	3.47	0.10	3.41	0.08	3.17	0.11	3.46	0.10	3.34	0.08	3.23	0.09	3.02	0.11	3.55	0.13	1.63	0.21	1.25	0.15	1.08	0.14	1.25	0.13
	3	3.22	0.11	3.12	0.11	2.87	0.09	3.20	0.11	3.60	0.17	3.19	0.08	3.01	0.09	3.43	0.12	1.56	0.18	1.07	0.27	0.91	0.11	1.20	0.22
	4	3.25	0.14	3.18	0.20	3.08	0.14	3.32	0.18	3.43	0.13	3.75	0.17	3.41	0.13	3.92	0.19	1.41	0.21	1.24	0.21	0.56	0.17	0.81	0.21
	5	3.53	0.16	3.52	0.17	3.38	0.10	3.26	0.11	3.72	0.17	3.86	0.16	3.65	0.15	3.65	0.15	1.59	0.20	1.35	0.22	1.22	0.11	1.03	0.22
	Total	3.38	0.06	3.39	0.06	3.16	0.05	3.28	0.06	3.47	0.06	3.43	0.07	3.24	0.06	3.51	0.07	1.43	0.09	1.19	0.10	0.87	0.07	1.00	0.09
B	1	3.76	0.10	3.87	0.14	3.94	0.13	4.02	0.09	4.12	0.15	4.12	0.13	4.26	0.10	4.38	0.07	2.03	0.17	1.84	0.16	1.41	0.23	1.63	0.23
	2	3.74	0.16	3.73	0.21	3.54	0.13	3.69	0.18	4.38	0.10	4.21	0.14	4.36	0.11	4.59	0.07	2.15	0.10	1.63	0.21	1.54	0.12	1.64	0.13
	3	3.78	0.09	3.68	0.09	3.77	0.08	3.69	0.10	4.28	0.05	4.15	0.07	4.31	0.05	4.27	0.07	1.80	0.15	1.49	0.17	1.40	0.17	1.25	0.22
	4	4.08	0.08	3.74	0.09	3.72	0.12	4.18	0.03	4.20	0.08	4.06	0.10	4.05	0.07	4.35	0.03	1.99	0.18	1.66	0.16	1.55	0.16	1.61	0.23
	5	3.22	0.05	3.10	0.10	3.23	0.07	3.86	0.09	3.46	0.04	3.36	0.08	3.50	0.06	3.93	0.14	1.16	0.19	0.58	0.17	0.81	0.18	0.94	0.13
	Total	3.71	0.06	3.62	0.07	3.64	0.06	3.89	0.05	4.09	0.06	3.18	0.06	4.09	0.06	4.30	0.05	1.83	0.09	1.44	0.10	1.32	0.08	1.42	0.09

* 10 pigs sampled at each visit.

Table 2. Distribution of bacterial counts at four sites on pig carcasses

Technique	Works	Site	No. of carcasses with counts (\log_{10}/cm^2)					
			3.0	3.0-3.49	3.5-3.99	4.0-4.49	4.5-4.99	5.0-5.49
TVC ₃₇	A	1	10	20	16	4	—	—
		2	19	20	5	5	1	—
		3	17	24	7	2	—	—
		4	0	28	9	2	1	—
	B	1	1	19	9	21	—	—
		2	—	17	8	7	3	—
		3	3	6	18	11	2	—
		4	—	9	18	21	2	—
TVC ₂₁	A	1	6	25	11	8	—	—
		2	5	30	6	7	2	—
		3	14	26	6	4	—	—
		4	6	26	10	4	4	—
	B	1	—	5	11	30	3	1
		2	—	9	14	21	5	1
		3	—	5	12	27	5	1
		4	—	2	2	34	12	—
<i>E. coli</i>	A		Nil	0-0.49	0.5-0.99	1.0-1.49	1.50-1.99	2.0+
		1	1	6	3	17	12	11
		2	3	6	12	13	10	6
		3	2	12	13	18	5	—
	B	1	—	7	21	10	5	5
		2	—	1	5	8	7	29
		3	1	4	10	12	11	12
		4	1	7	2	19	15	6
	4	2	3	6	15	14	10	

the visit effects were not consistent over all sites. When sites were compared, site 4 (cheek/jowl skin surface) TVC were significantly higher than those at the other three sites. Inspection of the data shows that the mean TVC, for all four sites, was lower at visit 5 than those obtained at the other four visits (Table 4).

At Abattoir A, site 2 was the most contaminated, but only marginally more so than site 1. At Abattoir B, site 4 was the most contaminated.

Counts at all four sites were significantly higher at Abattoir B than those at Abattoir A.

Total viable count at 21 °C

At both abattoirs, animals, visits and sampling site all had a significant effect on TVC value (Table 3). The values obtained at each sampling site were significantly affected by the day of sampling (significant interaction between visit and site).

When sites were compared at Abattoir A, site 3 (mid-dorsal region) TVC were significantly lower than those at sites 1 and 4, but not significantly different from those at site 2.

At Abattoir B, site 4 TVC were significantly higher than those obtained at the other three sites.

Table 3. Analysis of variance. *F* values for ANOVA terms

Bacterial count	Abattoir	Visit	Animal	Site	Visit × animal	Visit × site	Animal × site
TVC ₃₇	A	5.157**	4.435**	3.911* *	3.074**	1.024	0.681
	B	19.597**	2.149*	6.021**	1.995**	2.867**	0.637
	Combined	7.908**	2.984**	4.885**	3.271**	1.212	0.652
TVC ₂₁	A	18.228**	2.144*	3.918*	1.778*	2.589**	1.085
	B	70.747**	3.741**	16.697**	2.314**	1.943*	1.159
	Combined	10.523**	1.584	10.893**	2.151**	1.879*	0.928
<i>E. coli</i>	A	8.712**	5.267**	13.388**	3.530**	1.043	0.721
	B	33.542**	6.534**	16.391**	4.331**	0.947	1.355
	Combined	12.430**	2.402*	33.839**	3.311**	1.049	0.997
(Degrees of freedom)		(4)	(9)	(3)	(36)	(12)	(27)

* *F* value significant at *P* < 0.05.

** *F* value significant at *P* < 0.01.

Table 4. Comparison of sampling site mean bacterial counts on pig carcasses

Test...	TVC ₃₇		TVC ₂₁		<i>E. coli</i>	
	A	B	A	B	A	B
Abattoir...						
Site 1 v. site 2	ns	ns	ns	ns	**	**
Site 1 v. site 3	**	ns	**	ns	**	**
Site 1 v. site 4	ns	**	ns	**	**	**
Site 2 v. site 3	**	ns	ns	**	**	ns
Site 2 v. site 4	ns	**	ns	**	ns	ns
Site 3 v. site 4	ns	**	**	**	ns	ns

ns, No significant difference between site means at $P < 0.05$

** , Significant difference between site means at $P < 0.05$.

The most contaminated site at both abattoirs was site 4.

The TVC values at all four sites were significantly higher at Abattoir B than those at corresponding sites at Abattoir A.

E. coli counts

At both abattoirs animals, visits and sampling site all had a significant effect on TVC value (Table 3). The effect of sampling day was consistent over all sampling sites (no interaction between visit and site).

Comparison of sampling sites at Abattoir A showed that site 1 (dorsal to tail near anal opening) TVC were significantly higher than those at the other three sites. Site 2 TVC were significantly higher than those at site 3 but not significantly different from the TVC at site 4.

At Abattoir B, site 1 TVC were significantly higher than those at the other three sampling sites.

At both abattoirs site 1 was the most contaminated, producing the highest count on a carcass on over 50% of carcasses sampled.

E. coli counts at Abattoir B, at all four sites, were significantly higher than those at corresponding sites at Abattoir A.

Salmonella isolations

Details of salmonella isolated from pig carcasses sampled in the survey by site, visit and abattoir are presented in Table 5.

At Abattoir A, salmonellas were isolated from 6/50 pigs (12%) and from all sites except site 3. The isolation rate from site 4 was significantly higher when compared with site 2 (McNemar's $X^2 = 4.0$, $P = 0.04$) and site 3 (McNemar's $X^2 = 5.0$, $P = 0.03$), but not significantly higher than site 1. Four serotypes were isolated from pig carcasses at Abattoir A, *S. derby*, *S. give*, *S. virchow* and *S. ohio*. Only one serotype was isolated from each contaminated carcass, even when salmonellas were isolated from more than one site on a carcass.

At Abattoir B, salmonellas were isolated from 15/50 pigs (30%) and from all four sampling sites. Visit 4 contributed the greatest number of contaminated carcasses to this total as 9/10 carcasses yielded salmonellas of two serotypes. The salmonella isolation rate from site 4 was higher than at any other site but was not significantly greater than the isolation rate at these sites. Four serotypes were isolated at Abattoir B, these being *S. give*, *S. derby*, *S. infantis* and *S. meleagridis*.

Table 5. *Salmonella* isolations from pig carcasses

Abattoir	Visit	Sites				
		1	2	3	4	5
A	1	0/10	0/10	0/10	0/10	0/10
	2	1/10	1/10	0/10	2/10	2/10
	3	0/10	0/10	0/10	1/10	1/10
	4	1/10	0/10	0/10	2/10	3/10
	5	0/10	0/10	0/10	0/10	0/10
	Total	2/50 (4%)	1/50 (2%)	0/50	5/50 (10%)	6/50 (12%)
B	1	0/10	0/10	0/10	0/10	0/10
	2	0/10	0/10	0/10	1/10	1/10
	3	1/10	1/10	1/10	1/10	4/10
	4	8/10	6/10	7/10	9/10	9/10
	5	1/10	1/10	1/10	1/10	1/10
	Total	10/50 (20%)	8/50 (16%)	9/50 (18%)	12/50 (24%)	15/50 (30%)

The salmonellas isolated at Site 1 were *S. give* (8 isolations), *S. derby* (1 isolation) and *S. meleagridis* (1 isolation). At site 2 *S. give* was isolated 7 times and *S. derby* 3 times, while at site 3 *S. give* was isolated 7 times and *S. derby* 5 times. At site 4 *S. give* was isolated 10 times, *S. derby* 3 times and *S. infantis* once. *S. give* was isolated at all contaminated sites on visit 4, suggesting a single source of contamination. Two serotypes were isolated from 7 sites on 5 carcasses.

DISCUSSION

Sampling of carcass surfaces for microbiological contamination can be used to identify critical points in the slaughter and dressing process, (Gerats, Snidjers & van Logtestijn, 1981; Roberts, McFie & Hudson, 1980). Roberts, McFie & Hudson (1980) suggested that hygiene at slaughter can be assessed by using TVC incubated at 37 °C and that costs of testing can be reduced by sampling from carcass sites that were consistently 'dirty'. They also suggested that TVC incubated at 20 °C would assess the duration of chilled storage better than the TVC at 37 °C. Roberts and his co-workers suggested that adequate data from a range of countries are needed to determine the optimum sampling procedure for measuring carcass contamination.

A number of studies have looked at contaminated sites on pig carcasses and reached differing conclusions. Roberts, McFie & Hudson (1980) identified the distal hind-limb (trotter), lateral surface of the hind limb, lateral surface of the abdomen (belly) and mid-dorsal region as the pig carcass sites generally contaminated with high bacteria numbers of five abattoirs surveyed. Johanson *et al.* (1983) examined pig carcasses at nine abattoirs and found the cheek, lateral surface of the abdomen and the back of the neck to be the most contaminated sites. Scholefield, Menon & La (1981) found that the shoulder region was the most heavily contaminated carcass site. Reynolds & Carpenter (1974) found the lateral surface of the hind leg to be the most likely to be contaminated of three sites examined.

The two areas on pig carcasses most likely to be contaminated with bacteria are the neck and cheek region, and around the anus (Carpenter, Elliot & Reynolds, 1973). This contamination is most likely due to washings from the carcass in the former case, and faecal contamination in the latter case. Ingram & Roberts (1976) noted that heavily contaminated sites on beef carcasses may depend on processing and washing techniques. Roberts, McFie & Hudson (1980) found that there can be consistently dirty carcasses areas in abattoirs and that these areas may vary between abattoirs and between species.

In our survey four sites were examined to determine the most heavily contaminated sites, as measured by TVCs at 37 °C and 21 °C, *E. coli* counts and salmonellae isolations. The results showed that the cheek surface was the most contaminated site at two abattoirs when using the TVC incubated at 21 °C. Results of the TVC at 37 °C showed that the same site was the most contaminated at one abattoir, while the lateral surface of the hind limb had the heaviest contamination at the other abattoir. The cheek surface yielded the greatest number of salmonella isolations at both abattoirs. In contrast, *E. coli* counts were greatest on the area dorsal to the tail, near the anal opening.

The finding that the cheek surface was most frequently contaminated with salmonella and total aerobic microflora was not surprising. This area is the drain point for washings from the entire carcass and bacteria could be expected to accumulate in the area. The site which was most contaminated by *E. coli* is interesting and suggests that contamination of this area may occur from faeces voided from the rectum during processing. It appears that normal carcass washing procedures may have little effect on the reduction of *E. coli* at this site, suggesting that they may be firmly attached to the skin surface. The number of times that this site yielded the highest *E. coli* count at both abattoirs suggests that this area dorsal to the tail, near the anal opening is the best indicator of carcass contamination with *E. coli*.

On most individual visits there was little difference between sampling sites in TVC values or *E. coli* counts, highlighting the need to conduct extended sampling. This confirms the observations of Ingram & Roberts (1976) that heavily contaminated sites may vary from one occasion to another but that, on average, consistent differences in bacterial contamination between parts of a carcass may be identified.

Salmonellas were not constantly present on carcasses examined in this survey, with salmonellas not isolated from carcasses on some days. The differences in salmonella recovery rate between abattoirs may have been related to slaughter processes. Differences in the distribution of salmonellas on carcasses at Abattoir A are difficult to assess because of the small number of contaminated carcasses at this abattoir.

The consistent differences in the level of contamination at the two abattoirs demonstrate that it is possible to use microbiological techniques to compare the level of hygiene at different abattoirs.

Both Johanson *et al.* (1983) and Roberts, McFie & Hudson (1980) found significant interactions between abattoirs and the most heavily contaminated sites. Ingram & Roberts (1976) also noted that the most contaminated site is not necessarily the same for all abattoirs. The similar contamination patterns observed at the two abattoirs in this survey suggest that factors other than slaughtering

and dressing procedures may influence the sites where contamination is highest. The two abattoirs surveyed had different scalding techniques and markedly different dressing procedures.

This survey suggests that if the microbiological status of pig carcasses is being assessed, no single site may be appropriate. The differences in the recovery rate of *E. coli* and salmonella at various sites suggest that sampling from multiple sites may be required to assess the levels of different bacteria. Sampling sites must be considered when using *E. coli* numbers as an indicator for salmonella presence on pig carcasses. There appears to be no information on the sites most likely to be contaminated with *Clostridium*, *Yersinia* or *Campylobacter* species.

This survey also suggests that the hygiene of slaughtering and dressing of pig carcasses cannot be assessed on a single visit and that a number of visits are necessary to assess the hygiene of an abattoir.

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