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The effect of incubation temperature and site of sampling on assessment of the numbers of bacteria on red meat carcasses at commercial abattoirs

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SUMMARY

Several sites on commercial beef, pork and lamb carcasses were sampled at the end of the slaughterline. Total viable counts (TVC) of bacteria were assessed by incubation at 37, 20 and 1 °C in addition to presumptive coliforms (PC), Enterobacteriaceae (ENT) and faecal streptococci (FS). Statistical analyses showed consistently dirty sites within an abattoir but these sites varied from one abattoir to another. Inter-site differences were unaffected by the incubation temperature of the TVC. Numbers of PC, ENT and FS did not mimic TVC. Empirical sampling plans are proposed to detect the highest count on a carcass by bulking samples from known dirty sites. At the end of the slaughterline TVC 37 °C is the most useful bacteriological index.

INTRODUCTION

There is considerable current interest in the application of sampling plans to monitor numbers of bacteria on carcasses at several stages during their production and distribution. Bacterial numbers on the carcass surface at the end of the slaughterline may serve as a monitor of hygienic practice during slaughter and dressing; those after chilling might assess additionally the efficiency of refrigeration in the abattoir. Numbers after distribution would reflect the additional effects of distribution times and temperatures within a country or in international trade. However, there are few published bacteriological data on adequate numbers of commercial carcasses and there is a risk that future proposals, both for the numerical standards that should be achieved, and for the surveys (sampling plans) to monitor them, could be based on assumptions supported by little or no data. Following Ingram & Roberts (1976) there are several questions that should be answered before any satisfactory sampling scheme can be designed:

(1) In view of the possible application of quality-control rejection procedures, is the distribution of bacterial numbers, as reflected by the logarithmic total viable count, normally distributed?

(2) Within an abattoir are there consistently dirty (in a bacteriological sense) anatomical sites on the carcass of a particular species?

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Table 1. *Species and abattoirs sampled, and bacteriological tests performed*

Species	Abattoir	TVC 01	TVC 20	TVC 37	PC	ENT	FS	No. of carcasses
Pig	A1	√	√	√	√	√	√	10
	A2	√	√	√	√	√	√	14
	B	√	√	√	√	√	√	10
	C		√	√	√	√	√	6
	D		√	√	√	√	√	6
Beef	A	√	√	√	√	√	√	10
	B	√	√	√	√	√	√	10
	E		√	√	√	√	√	6
Lamb	A 1	√	√	√	√	√	√	10
	A 2	√	√	√	√	√	√	8
	D		√	√	√	√	√	6
	F1		√	√	√	√	√	6
	F2		√	√	√	√	√	6
	G		√	√	√	√	√	6
	E		√	√	√	√	√	6
	H		√	√	√	√	√	6

TVC 01 = total viable count incubated at 1 °C; TVC 20 = total viable count incubated at 20 °C; TVC 37 = total viable count incubated at 37 °C; PC = presumptive coliform; ENT = Enterobacteriaceae; FS = faecal streptococci.

(3) Are these sites the same when bacterial numbers are assessed at different incubation temperatures?

(4) Are there sites that are consistently dirty across a number of abattoirs?

(5) How far do counts on specific organisms, such as presumptive coliforms (PC), Enterobacteriaceae (ENT) or faecal streptococci (FS), reflect the behaviour of total viable counts (TVC) as defined under (1) to (4) above.

This paper reports the results of a large experiment on commercial pork, beef and lamb carcasses designed to answer these questions. The implications of these results to some possible sampling schemes are discussed.

MATERIALS AND METHODS

Design

Counts of PVC, PC, ENT and FS were taken from samples that varied in origin of site, animal, incubation temperature and abattoir. Full details of the sampling design appear in Table 1, and the anatomical sites used for each species in Table 2.

Sampling

Carcasses were sampled by swabbing the 100 cm^2 areas (listed in Table 2) with sterile cotton wool using the wet and dry swab technique of Kitchell, Ingram & Hudson (1973). Swabs from each site were taken into 10 ml of maintenance medium (0·85 % saline + 0·1 % peptone) in wide-necked, screw-capped universal containers each of 28 ml capacity, which were then shaken on a vortex mixer ('Rotamixer', Hook & Tucker Ltd) for 30 s.

Table 2. *Sampling sites* on pigs, beef or lamb*

Site no.	Pig	Beef	Lamb
1	Distal hind limb (trotter)	Neck	Leg, lat.
2	Hind limb, lat.†	Brisket	Leg, med.
3	Abdomen, lat. (belly)	Fore-rib	Abdomen (flank)
4	Mid-dorsal region (mid-back)	Flank	Thorax, lat.
5	Back of neck	Sirloin	Neck, lat.
6	Throat	Flank, groin	Crutch
7	Cheek	Rump	Breast, lat.
8	Thorax, lat.	Round, lat.	Diaphragm, thoracic surface
9	Leg, med.‡	Round, med.	Dorsal region (back)
10	Abdomen, med.	Flank, med.	Thorax, med. (pleural cavity)
11	Thorax, med.	Sirloin, med.	
12	Neck, med.	Brisket, med.	
13	—	Fore-rib, med.	

* Each carcass in Table 1 was sampled at all the sites listed for that species in Table 2.
† lat. = lateral surface.
‡ med. = medial surface.

Counting

Manufactured Pt/Ir loops (0·6 mm diameter welded to 6 mm internal diameter) of about 0·02 ml capacity replaced the traditional 1 ml pipette, and nine times the loop volume of sterile diluent was used for the dilution blanks in sterile stainless-steel tiles. Decimal dilutions were prepared, flaming the loop between dilutions, and duplicate samples of each spread on the surface of Plate Count Agar (Oxoid CM 325) + 1 % NaCl pre-dried at 50 °C in 9 cm diameter Petri dishes, using a quarter of the agar surface for each dilution. After incubation at 37 °C (2 days), 20 °C (4 days) or 1 °C (14 days) viable counts were calculated from the numbers of colonies at two dilution levels by the method of Farmiloe *et al.* (1954).

Presumptive coliforms (PC) were enumerated on MacConkey's Bile Lactose Agar without salt (Oxoid CM 7b) incubated at 37 °C for 24 h counting typical large, dark pink non-mucoid colonies. Enterobacteriaceae were counted in over-layed pour plates of Violet Red Bile Agar (Oxoid CM 107), to which 1 % glucose was added, incubated for 37 °C for 24 h. Typical dark red colonies surrounded by a reddish halo were counted. Faecal streptococci were counted on Kanamycin Aesculin Azide Agar (Oxoid CM 481) incubated at 37 °C for 24 h, counting colonies with black haloes.

For the statistical analysis all counts were expressed as logarithms to the base 10. Counts of 1 or less were transformed to zero on that log scale.

Statistical methods

All analyses were applied to each animal species separately.

Testing for normality. A probability plotting technique was used (Barnett, 1975) to test for normality of TVC counts at each incubation temperature at each site. Counts were ranked and each count plotted against the standardized normal

deviate for which the area to the left under the normal curve was $i/(n+1)$, where i is the rank of that count and n is the total number of counts. If the values are normally distributed the points should lie on a straight line with intercept equal to the mean, and slope equal to the standard deviation.

Total viable counts

A three-factor analysis of variance was performed on each species to test the interdependence and the overall effects of different anatomical sites, temperatures and abattoirs.

For those not familiar with this technique, the first step is to test whether the interactions between these factors are statistically significant. A significant interaction, e.g. between temperature and site, would indicate that the differences in count between the sites were not the same at different incubation temperatures. Conversely, lack of interaction, e.g. between site and abattoirs, would imply that the differences (in bacterial numbers) between the sites were not significantly different between the abattoirs, i.e. any observed differences between sites were maintained regardless of abattoir sampled.

Where a significant interaction was observed the differences between the levels of one of the factors must be studied at each level of the other factor to identify the differential effects. For example, if a significant SITES × TEMPERATURE interaction had been recorded, it would be necessary to compare the mean counts for each site at each incubation temperature.

This analysis was applied initially to the two abattoirs where all three incubation temperatures were used (Analysis 1). The 1 °C counts were then excluded and the analysis applied to all the abattoirs (Analysis 2).

Specific counts, PC, ENT, FS

The large number of zeros (counts of 1 or less) in these data prevented the use of analysis of variance. Instead, the absolute counts were grouped into three mutually exclusive categories: 1 or less, 10 or less, greater than 10. The numbers of counts in each category were then classified by anatomical site and abattoir. A contingency table analysis to test the existence of differential distributions of counts among sites or abattoirs was performed using the GLIM computer program (Nelder & Wedderburn, 1972).

RESULTS

The normal order plots for TVC data indicated that, with minor aberrations, the assumption of log normality was valid. Table 3 lists overall mean values for TVC, the mean TVC at 1, 20 and 37 °C, the maximum and minimum values and the sites at which they were obtained.

Table 4 summarizes the significant effects and interactions found for Analyses 1 and 2 for the three species. The following important points emerge:

(1) The SITES × TEMPERATURE interaction term never reached the 5% significance level, implying that the inter-site differences are maintained no matter

Table 3. *Bacterial counts on commercial carcasses*

Species	Abattoir code	No. carcasses	Overall mean†	Residual SD‡	Temperature means averaged over sites			Pooled SED	Site means averaged over temperature				Pooled SED
					1 °C	20 °C	37 °C		Site§	Max. (value)	Site§	Min. (value)	
Pig	A 1	10	3·84	0·39	2·65	4·03	3·65	0·07	1	(4·19)	9	(3·44)	0·11
	A 2	14	4·14	0·47	2·21	4·22	4·06	0·05	1	(4·62)	11	(3·07)	0·11
	B	10	3·26	0·44	2·03	3·21	3·32	0·07	1	(3·49)	11	(2·92)	0·11
	C	6	2·16	0·47	—	2·11	2·20	0·10	10	(2·94)	11	(1·60)	0·11
	D	6	2·76	0·58	—	2·75	2·76	0·10	3	(3·84)	11	(1·86)	0·11
Beef	A	10	2·90	0·52	1·96	3·12	2·68	0·14	2	(3·42)	11	(2·12)	0·24
	B	10	2·96	0·53	1·83	2·95	2·98	0·14	4	(3·45)	13	(2·59)	0·24
	E	6	1·93	0·52	—	2·03	1·83	0·10	2	(2·58)	10	(1·30)	0·24
Lamb	A 1	10	3·31	0·50	2·45	3·45	3·16	0·10	7	(3·68)	10	(2·59)	0·26
	A 2	8	3·32	0·47	1·98	3·44	3·20	0·11	7	(3·91)	10	(2·36)	0·26
	D	6	3·07	0·57	—	3·13	3·01	0·12	6	(3·94)	10	(2·36)	0·26
	E	6	2·60	0·43	—	2·67	2·54	0·12	3	(3·21)	10	(1·98)	0·26
	F 1	6	2·81	0·75	—	2·87	2·76	0·12	4	(3·27)	1	(1·88)	0·26
	F 2	6	2·92	0·76	—	2·94	2·91	0·12	6	(4·52)	1	(1·77)	0·26
	G	6	3·28	0·44	—	3·32	3·25	0·12	7	(3·79)	10	(2·44)	0·26
	H	6	2·94	0·50	—	3·13	2·76	0·12	9	(3·58)	10	(2·31)	0·26

† The overall mean is for TVC 37 and TVC 20 only.

‡ Residual standard deviation after temperature and site effects have been eliminated.

§ Site, see Table 2.

Table 4. *Summary of significance of main effects: abattoirs* (A), *temperatures* (T), *sites* (S) *and corresponding interactions*

Species	Analysis	T × S	A × S	A × T	S†	T	A
Pig	1	NS	NS	***	***	*	NS
	2	NS	***	***	**	NS	***
Beef	1	NS	*	***	*	*	NS
	2	NS	***	***	*	NS	NS
Lamb	1	NS	NS	***	***	*	NS
	2	NS	***	NS	***	***	**

NS = not significant at $P < 0{\cdot}05$.
* = $P < 0{\cdot}05$.
** = $P < 0{\cdot}01$.
*** = $P < 0{\cdot}001$.
† If interactions are significant, corresponding main effects are tested against interaction mean square.

which incubation temperature is used for the bacterial count. Altering the incubation temperature predictably changed the apparent numbers of bacteria with counts at 1 °C lowest, those at 37 °C intermediate, and at 20 °C equal to or greater than those at 37 °C (Table 3).

(2) In Analysis 1 only the beef counts revealed a significant interaction between ABATTOIRS and SITES. However, when more abattoirs were included in Analysis 2 each species showed a highly significant ABATTOIR × SITES interaction. We may thus infer that, within an abattoir, there may be sites which are consistently dirty, but that these dirty sites vary in different abattoirs. To examine the extent of this variation, Table 5 shows the ranking of each site mean within a visit to an abattoir. As high ranks correspond to 'bacteriologically dirty' sites it is clear that some sites are consistently 'dirtier' than others and Table 6 lists the sites for each species that most frequently showed the maximum count on a carcass.

Contingency table analysis of specific counts revealed a differential pattern of counts in the three categories among abattoirs, and to a much lesser extent among sites, for the following data sets:

Pig	Presumptive coliform, Enterobacteriaceae
Beef	None (FS was not tested because most sites carried none)
Lamb	Presumptive coliform, Enterobacteriaceae, faecal streptococci

Finally, scatter diagrams of TVC counts were plotted against Enterobacteriaceae, presumptive coliforms and faecal streptococci and inspected visually for any relationship. No clear trends were evident and for reasons of space no plots are presented here.

DISCUSSION

Presumptive coliforms, Enterobacteriaceae and faecal streptococci

Considering the results of the specific counts PC, ENT and FS in pig and lamb, significant differences in PC and ENT counts existed between abattoirs, although

Table 5. *Ranking*† *of sites by bacterial count on commercial carcasses to indicate abattoir × site interactions*

	Pig						Beef				Lamb								
	Abattoir						Abattoir				Abattoir								
Site‡	A1	A2	B	C	D	Overall	A	B	E	Overall	A1	A2	D	F1	F2	G	E	H	Overall
1	12	12	12	4	8	12	6	2	6	5	7	7	5	1	1	6	3	5	3
2	5	11	8	9	2	7	13	11	13	13	4	5	9	6	8	4	6	3	6
3	4	7	11	7	12	11	9	5	10	9	8	9	6	9	6	7	10	9	9
4	11	6	10	10	4	6	12	13	4	12	9	6	7	10	5	9	9	8	8
5	9	9	6	11	3	9	5	8	11	6	3	4	3	8	7	8	7	6	5
6	8	5	3	5	11	5	10	12	9	10	5	8	10	4	10	3	8	4	10
7	7	10	5	2	10	10	7	9	8	7	10	10	8	7	3	10	5	7	7
8	10	8	7	3	9	8	8	4	12	11	2	3	4	5	9	2	2	2	2
9	1	4	4	6	5	3	11	10	7	8	6	2	2	3	2	5	4	10	4
10	6	3	9	12	7	4	2	6	1	2	1	1	1	2	4	1	1	1	1
11	3	1	1	1	1	1	1	3	5	1	.	.	.	.	.	.	.	.	.
12	2	2	2	8	6	2	3	7	3	4	.	.	.	.	.	.	.	.	.
13	.	.	.	.	.	.	4	1	2	3	.	.	.	.	.	.	.	.	.

† The site with the lowest bacterial count is ranked '1', and the highest count '12' (pig), '13' (beef) and '10' (lamb). Ranking was performed on means of TVC 37 and TVC 20 only.

‡ See Table 2.

Table 6. *The sites most consistently contaminated by high bacterial numbers*

Pig		Beef		Lamb	
Distal hind limb (trotter)	(1)	Brisket	(2)	Abdomen (flank)	(3)
Hind limb, lat.	(2)	Forerib	(3)	Thorax, lat.	(4)
Abdomen, lat. (belly)	(3)	Flank	(4)	Crutch	(6)
Mid-dorsal region (mid-back)	(4)	Flank groin	(6)	Breast, lat.	(7)
Abdomen, med.	(10)	Round, lat.	(8)		

the day-to-day variation in counts has not been estimated. Variation among sites is less important and consistently 'dirty' sites were not evident from the contingency tables. The lack of difference between abattoirs in beef is probably due to the low level of occurrence of the organisms tested for, none being detected on many of the sites tested, and to the fact that only three abattoirs were surveyed. Combining these results with the failure of these specific organisms to mimic the behaviour of TVC seems to rule out their use as a substitute for TVC in quality control sampling.

Total viable count

The results for TVC, in contrast, have considerable implications for those intending to design sampling schemes. The lack of interaction between sites and incubation temperature implies that counts at one temperature only may be required; 37 °C would be the most convenient since it requires the shortest incubation time.

The number of significant interactions between abattoirs and both sites and incubation temperature implies that it is not possible to sample just one site or to use only one incubation temperature without subsequent loss of precision. However, in the case of quality control the aims narrow to detecting the highest count on a carcass. The data have been used to compare three empirical sampling schemes designed to detect the highest count on a carcass.

In order of decreasing cost to perform, the schemes are:

(a) Obtain a total viable count for each of the sites used in the experiment (as given in Table 2) and characterize the carcass by the maximum TVC obtained.

(b) For each species a subset of sites that most consistently included the maximum TVC count on a carcass was selected. (Note that these are not necessarily the sites showing the highest *mean* TVC counts.) Each carcass is then characterized by the maximum TVC count observed in this subset.

(c) Using 'dirty sites' only, obtain a swab from each site combine these swabs and make one TVC of this single sample. This scheme was simulated on the data by calculating the average of the absolute counts.

Within the framework of the experiment sampling scheme (and the precision of counting bacteria) (*a*) is, of course, 100 % accurate. However, practical interest centres on how closely schemes (*b*) and (*c*) can reproduce these results. To compare the schemes the values obtained using schemes (*b*) and (*c*) on each carcass were compared on the basis of being within one or two doublings of the scheme (*a*) value (overall maximum), i.e. a difference of less than 0·3010 on the $\log_{10}$ counts indi-

Table 7. *Number of carcasses/total number of carcasses for which maximum counts of schemes (b) and (c) are within prescribed doublings of overall maximum count on carcass*

		37 °C			20 °C		
Scheme ...		(b)	(c)	(c)	(b)	(c)	(c)
Doubling ...		<1	<1	<2	<1	<1	<2
Species	Abattoir						
Pig	A 1	10/10	4/10	10/10	10/10	3/10	10/10
	A 2	4/5	1/5	5/5	3/5	1/5	3/5
	B	9/10	4/10	8/10	10/10	1/10	10/10
	C	5/6	1/6	6/6	5/6	0/6	1/6
	D	6/6	1/6	6/6	4/6	1/6	4/6
Beef	A	9/10	5/10	9/10	9/10	4/10	9/10
	B	8/10	2/10	8/10	7/10	3/10	7/10
	E	5/6	1/6	5/6	6/6	1/6	4/6
Lamb	A 1	8/10	4/10	7/10	8/10	4/10	8/10
	A 2	8/8	5/8	8/8	8/8	4/8	8/8
	D	6/6	3/6	6/6	5/6	3/6	5/6
	E	6/6	2/6	6/6	6/6	2/6	6/6
	F 1	3/6	1/6	3/6	5/6	1/6	5/6
	F 2	6/6	0/6	6/6	4/6	1/6	5/6
	G	5/6	2/6	5/6	5/6	0/6	4/6
	H	5/6	5/6	5/6	4/6	1/6	3/6

cates that doubling the lower value would give a value in excess of the higher value, etc.

Considering first the performance of scheme (*b*) relative to (*a*) using a TVC at 37 °C, on 15 of 16 abattoir visits 80 % or more of the maximum carcass counts at a visit were within one doubling of the overall maximum count, while using the 20 °C TVCs on 11 of 16 occasions attained that level (Table 7).

Bulking the swabs (Table 7) and still using less than one doubling as the criterion (scheme (*c*)) loses sensitivity, but detection to within two doublings almost restores the level of sensitivity achieved by examining the 'dirty' sites separately (i.e. using TVC 37 °C on 14 of 16 occasions and using TVC 20 °C on 10 of 16 occasions) and involves a considerable reduction in effort.

Within international trade there is sometimes a wish to categorize individual carcasses, or batches of carcasses, by a bacteriological test, to assess the level of hygiene under which they have been produced, or their bacteriological 'quality'. So few data have been published that there is a danger that the choice of any numerical criterion may not be statistically based. Our considerable amount of data on commercial carcasses suggests that TVC is the most useful bacteriological index, and that the hygiene of slaughter may be assessed by incubating the TVC at 37 °C, since it requires a shorter incubation period than at lower temperatures and loses no information. Many bacteria which grow under chill storage are unable to grow at 37 °C, therefore the duration of chill storage would probably be better reflected by TVC 20 °C (or TVC 25 °C). Although bacteria are unevenly distributed

over carcasses, there were relatively systematic 'dirty' areas. If effort is concentrated on those areas, the costs of bacteriological testing can be reduced, but those areas must first be defined in each abattoir. Similarly, effort and costs can be cut by bulking swabs, with some, but not necessarily important, loss of sensitivity.

Before bacteriological tests on carcasses are applied with a view to acceptance or rejection, more extensive data of the type illustrated above are required, preferably from more commercial abattoirs, in different seasons and in different countries.

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