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HYGIENE IN MEAT PROCESSING PLANTS.

II. Methods of assessing carcase contamination

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2. Methods of assessing carcase contamination.

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INTRODUCTION

Before a bacteriological examination of a food can begin, the problem of how to sample arises. This is often difficult, and particularly so with animal carcases where the spread of surface contamination is not even, may vary from carcase to carcase, and from abattoir to abattoir. It is also important to know if the pattern of bacterial contamination acquired during butchering persists during refrigerated storage, and if areas most heavily contaminated at first spoil most rapidly. If the areas of rapid spoilage were known steps could be taken in the abattoir to reduce this by better butchering and washing techniques. The bacterial types present are also important.

Different workers have used different techniques when sampling animal carcases. Haines (1933) used a small platinum loop of known area to sample the slime on carcases stored 1 week at 5-0°C, where very large numbers of bacteria were present (up to 10^9 sq cm). This worker (Haines, 1937) also developed a method whereby a sterile cork-borer was pressed vertically into the tissues to a depth of a centimetre or so and from this a disc of about 2 mm thick was cut from the surface. The bacteria on the surface were then removed by shaking vigorously with glass beads and sterile saline. A thicker disc could be ground up mechanically using a stirrer and sterile sand. It was necessary to take sufficient samples to minimise the large experimental errors involved. The greatest objection however to the method is the damage to the surface of the carcase. It is more suited to the sampling of minced or sliced meat. Empey and Scott (1939) used a very similar method, and ground the excised disc of tissue in a mortar with sterile sand and saline. On sides of beef they sampled muscle in the aitch bone area, the neck on both the vertebral and ventral sides of the jugular furrow, and the sternum muscle. They estimated the bacterial populations on fat and

connective tissue of the buttock, rump, loin, rib and shoulder areas.

More recently, Walter (1955) recorded that non-destructive sampling of meat could be carried out by placing a nutrient soaked membrane-filter on the surface to be tested. For quantitative work this filter could be desintegrated in a dilution bottle containing glass beads or in a Waring blendor. No experimental date were given. Angelotti and Foter (1958) developed a direct surface agar plate for detecting bacterial contamination on non-porous surfaces. The direct contact method has been simplified by ten Cate (1963) by using nutrient agar or a selective medium in artificial casings, which allows a large number of "impressions" of a surface to be taken quickly. This method (which is a development of the contact plate method of Walter and Hucker (1941) is of great use where relatively smooth surfaces can be sampled, such as machinery, instruments, tables, chopping blocks, walls, floors, etc., after cleaning has been carried out. Such a method loses much of its usefulness when bacterial numbers rise beyond 30-50 per sq cm since it is not possible to count easily individual bacterial colonies when numbers are higher than this. It is not satisfactory on a rough surface such as a side of beef, which is often fairly heavily contaminated.

Another approach is that of Dyett (1963) who used a sterile scalpel to scrape the surface of the meat, and then carried out a direct microscope count or a total viable count on the scrapings. This method has the advantage of not damaging the carcase.

Clark (1965) has used a rinsing technique with a known volume of sterile liquid under a constant pressure to sample poultry skin. This method appears promising, but requires special equipment.

In this laboratory the method at first used was similar to that of Dyett (1963). As much as possible of the surface of the sides of the carcase was scraped with a sharp knife to give 2-3 g of scrapings. These were then examined to obtain the total bacterial count per g of surface scraping. It soon became obvious that contamination varied greatly on different areas of the carcase, and it was realised that more critical work was required to determine

the spread of such contamination. In addition, slaughtering techniques vary from one abattoir to the next and it was thought necessary to carry out the work in at least two different types of abattoir. A further aim was to find whether initially heavily contaminated areas continued to be so after the carcase was cooled and during refrigerated storage, and if such areas were likely to spoil more quickly than less contaminated areas.

EXPERIMENTAL

Cattle carcases.

To estimate the spread of contamination on cattle carcases two abattoirs were selected. One was large and modern with a complete "on-the-rail" system of butchering (abattoir A); the other was older and small, and had an incomplete system, the animal being laid on the floor for partial hide-removal (abattoir B). Seven sites were selected on each side of the carcase, viz on the (i) hindleg, (ii) rump, (iii) thin flank, (iv) sirloin, (v) brisket, (vi) foreleg, (vii) neck. To sample, a 16 sq cm area on each of the sampling sites was outlined with a sterile metal template. Then a small sterile cotton-gauze swab (4 cm of cotton-gauze surgical bandage on a 11.5 cm wooden applicator) was rubbed for 15 sec over the enclosed area with moderate pressure. The swab was then broken into a sterile universal Macartney bottle and transported to the laboratory for examination. On some carcases an adjacent area of 16 sq cm on each sampling site was swabbed with a Ca alginate swab (50 mg alginate on a wooden applicator) for 15 sec to see if this type of swab would give a higher recovery of bacteria. To determine how many swabs were necessary to remove most of the bacteria from such areas, certain other areas of muscle and fatty tissue were swabbed with 5 separate cotton-gauze or alginate swabs. The effect of using very large swabs was studied by swabbing certain 15 x 15 cm areas of several carcases with 7.5 cm of cotton-gauze surgical bandage attached to wooden skewers. When sampling cooled dry carcases the swabs were moistened in sterile 0.5 per cent peptone water prior to use.

In the laboratory the swabs were shaken for 10 min on a mechanical shaker in 0.5 per cent sterile peptone water, or in the

case of the alginate swabs in quarter strength Ringer solution with 1 per cent of Na hexametaphosphate added. Further decimal dilutions were made in 0.5 per cent peptone water or quarter strength Ringer solution. Three 0.02 ml replicate drops from each of three dilutions were plated, by the method of Davis and Bell (1959) on nutrient agar. The composition of this medium was: peptone (Oxoid L37) 10 g; Lab-Lemco 10 g; NaCl 5 g; agar (Oxoid no.3) 12 g, in 1,000 ml deionized water, the medium adjusted to pH 7.4 and sterilized for 15 min at 15 lb (121°C). The inoculated plates were incubated for 3 days at 22°C and the colonies counted from a suitable dilution.

Sheep carcases.

These were sampled prior to cooling, using the metal template technique. The sites sampled were on the (i)hindleg, (ii) rump, (iii) flank, (iv) brisket, (v) foreleg, (vi) neck and on some carcases (vii) crutch and (viii) belly. Three 4 cm cotton-gauze swabs were employed to swab each 16 sq cm area. Otherwise the procedure was similar to that used with cattle carcases.

RESULTS

Cattle carcases.

It was necessary first of all to establish which type of swab to use, and how many swabs per area. Some results are given in Table 1, of repeated swabbing of 16 sq cm areas of the surface of carcases. As might be expected only a proportion of the bacteria recoverable by this method were removed by the first swab. In fact the recovery obtained by the first swab as a percentage of all five varied from 57 - 93% depending on the type of surface and type of swab. Table 2 gives data from the comparison of cotton-gauze and alginate swabs and also from the use of triple swabs on the same area. It seems that if the highest possible recovery is required, more than one swab should be used. If only one swab is to be employed then it should preferably be of alginate composition. However if three swabs per area are employed, the cotton-gauze is probably equally good. In most of the subsequent work reported a single cotton-gauze swab was employed for several reasons. When a large number of areas had to be swabbed quickly, the single cotton-gauze was more convenient. It was a much

stronger swab, and less liable to break when in use; it could also be wrapped and sterilized individually. Very large swabs used on large areas gave a poorer recovery.

The results obtained from the examination of areas on different sampling sites on cattle carcases are given in Table 3. Of those sites sampled, the brisket region was generally the most heavily contaminated, followed by rump (or sirloin) and foreleg. In Table 4 are listed values obtained in a comparison between four 16 sq cm areas on the brisket on each side of twelve carcases and the other sampling sites on the same carcase, at abattoir A. Obviously this is one region which will require special treatment to reduce the contamination level.

Of the sites sampled on the carcase, three (rump, sirloin and brisket) were fatty tissue, whereas the remainder were largely muscle. A comparison of levels of contamination on fatty and muscle tissues is given in Table 5. These results indicate that the fatty tissues, even when the brisket is excluded from the comparison, are those carrying the most bacteria and will therefore need most attention when washing the carcase. These are often the more difficult parts to wash properly.

Only small differences were found between contamination on the left side of the carcase compared with that on the corresponding right side.

It is important to know if sites which carry most initial contamination will remain so after cooling, and if these will tend to spoil more quickly than sites less heavily contaminated. The answer to this can only be found by a close study of the bacterial types involved at various stages during refrigerated storage. The results given in Table 6 from 15 carcases at abattoir A, however, indicate that those sites heavily contaminated during butchering will continue to be so during refrigerated storage (at 2-4°C). Probably these will spoil more rapidly than less contaminated sites.

Sheep carcases.

Less work has been done on sheep carcases, but Table 7 summarizes some of the results obtained from abattoir A. Again, the brisket, rump and foreleg appear to be the most heavily contaminated during

butchering. In this case three swabs were used per area swabbed, and in a replicate swab trial it was found that three such swabs recovered over 90 per cent of those bacteria which could be recovered by five replicate swabs. Fatty tissues were more heavily contaminated than muscle tissues.

DISCUSSION

The data obtained in these experiments are being subjected to a statistical analysis which is as yet incomplete. However, it has been possible to suggest bacteriological standards which should be attainable if reasonably good butchering techniques are employed. These are as follows:

Site sampled	Cattle carcases (freshly butchered) Total count on nutrient agar/sq cm after 3 days at 22°C not to exceed approx:
Rump	3,000
Brisket	10,000
Foreleg	3,000

Similar standards have been suggested for sheep. In practice, under conditions of good hygiene, these standards could be a good deal more stringent, but they do offer a yardstick by which butchering hygiene can be assessed. There should be little increase in these counts after several days in refrigerated storage at 2-4°C.

SUMMARY

Sampling methods for surfaces are discussed. Experiments are described the aim of which was to develop a suitable sampling technique for freshly butchered cattle and sheep carcases, and carcases held in refrigerated storage. The method adopted was to swab a 16 sq cm area on each of the rump, brisket and foreleg regions of the carcase with a single small cotton-gauze swab. The bacteria from this swab were recovered on nutrient agar incubated for 3 days at 22°C. Bacteriological standards based on the results have been formulated.

TABLE 1 (A2)

Recovery of bacteria by replicate swabs on muscle and fatty tissues of cattle carcases.

No. of areas	Type of surface	Type of swab	Log bacterial number per sq cm recovered by swab no				
			1	2	3	4	5
14	Muscle	Cotton-gauze	3.04	2.13	2.04	1.90	1.52
14	Muscle	Alginate	3.35	2.64	2.16	2.00	1.64
5	Fatty	Cotton-gauze	3.91	2.14	1.68	1.54	2.62

TABLE 2

Recovery of bacteria by cotton-gauze and alginate swabs from cattle carcases.

No. of carcases	Swabbing method	Log bacterial number recovered per carcase (14 areas) using swabs of	
		Cotton-gauze	Alginate
11	Single swab	5.77	6.11
3	Triple swab	5.66	5.62

TABLE 3 (A2)

Contamination on different sampling sites on cattle carcases.

No. of carcases sampled	Abattoir	Sampling method	Log bacterial number per sq cm, recovered from Hindleg	Rump	Flank	Sirloin	Brisket	Foreleg	Neck
39	A	Cotton-gauze single swab	3.08	3.48	2.45	3.10	4.04	3.17	2.72
3	A	Cotton-gauze triple swab	2.60	3.21	2.68	3.77	3.63	3.17	2.70
10	B	Cotton-gauze single swab	2.71	3.35	2.97	3.51	3.79	3.03	2.62

TABLE 4

Contamination on brisket sampling site of cattle carcases.

Carcase No.	Log bacterial number per sq cm, recovered from 4 areas on brisket on left side of carcase	4 areas on brisket on right side of carcase	12 other areas of carcase
1			
2	3.63	3.67	2.86
3	3.56	3.78	2.91
4	4.10	3.71	2.40
5	3.56	3.10	3.21
6	4.54	4.09	3.26
7	4.34	4.11	3.31
8	3.84	3.18	2.50
9	4.28	3.50	2.61
10	3.70	3.37	2.59
11	4.08	3.89	2.91
12	3.24	3.03	3.03
	3.87	3.71	2.88

TABLE 5 (A2)

Contamination on fatty and muscle tissues of cattle carcases.

No. of carcases sampled	Abattoir	Sampling method	Log bacterial number per sq cm, recovered from: Fatty tissue	Fatty tissue excluding brisket	Muscle tissue
39	A	Cotton-gauze single swab	3.70	3.32	2.94
3	A	Cotton-gauze triple swab	3.59	3.56	2.85
10	B	Cotton-gauze single swab	3.59	3.43	2.87

TABLE 6

Effect of refrigerated storage on bacterial contamination of cattle carcases

No. of carcases	Storage time (days)	Log bacterial number per sq cm, recovered from: Hindleg	Rump	Flank	Sirloin	Brisket	Foreleg	Neck
5	1	3.27	3.40	2.83	2.99	4.96	4.19	3.40
5	2	2.57	3.51	2.51	2.71	5.28	4.92	3.28
5	7	3.81	4.47	3.39	4.79	> 6.09	5.55	4.62

TABLE 7 (A2)

Contamination on different sampling sites on sheep carcases.

No. of carcases sampled	Log bacterial number per sq cm, recovered from							
	Hindleg	Rump	Flank	Brisket	Foreleg	Neck	Crutch	Belly
20 (freshly butchered)	3.63	4.03	3.92	4.35	4.25	3.23	3.65+	3.81+
3 (2 days in chillroom)	4.16	4.30	4.26	4.80	4.27	4.04	3.82	4.29

+Values for 12 carcases.

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