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Microbiological methods, sampling plans and criteria for red meat abattoirs in the context of HACCP/QA (M01020)

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Project overall aim and the relevance for the Food Standards Agency:

The proposed research project would provide the FSA and the industry with scientific basis for recommendations to the red meat industry related to methods for the microbiological testing of red meats; related sampling plans; and hygiene performance criteria in the context of HACCP/QA.

Project overall work plan:

01 Objective - Determine the appropriateness, if any, of the HACCP plans in red meat abattoirs including the microbiological sampling programmes and criteria used.

02 Objective - Experimentally determine performance of carcass sampling (wet-dry swabbing) method from the FSA protocol and it's equivalence to the EU reference (excision) method.

03 Objective - Experimentally determine performance of common microbiological method(s) used by the meat industry.

04 Objective - Determine field-performance of carcass sampling (wet-dry swabbing) method from the FSA protocol and it's equivalence to the EU-reference (excision) method under commercial conditions.

05 Objective - Develop sampling plans/performance criteria for red meat abattoirs based on validated methods from the FSA protocol.

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EXECUTIVE SUMMARY

In the first stage of the project i.e. before the start of the experimental and the field work could commence, it was necessary to obtain information from the UK meat industry on the current status of affairs with respect to sampling method(s) for microbiological testing of red meats of proven acceptability to the industry; sampling plans for high- and low-throughput red meat operators; and hygiene performance criteria in the context of HACCP/QA. A postal survey of slaughterhouses in England licensed under the Fresh Meat (Hygiene and Inspection) Regulations 1995 (As Amended) was conducted from 16 July to 16 August 2002. Quality Assurance department of red meat abattoirs were asked about their HACCP systems in place, staff hygiene training, details on sampling plans, sampling analysis, and laboratory methods and reporting. The total response rate for the survey was 10% (31/299). This relatively low response rate may not be unexpected for a postal survey of the red meat plant community given the sensitive nature of verification of HACCP and food hygiene as a subject matter. Among the red meat plants that responded, approximately two-thirds were full throughput plants (65%; 21/31), and 35% (11/31) were low throughput plants. The number of animals that full throughput plants processed per week ranged from 30 to 13,000 (mean = 4267 animals). The number of animals that low throughput plants processed per week ranged from 10 to 2,200 (mean = 331 animals). Over three-quarters of the meat plants processed cattle (77%; 24/31), 74% (23/31) processed sheep/lamb, a quarter (25%; 18/31) processed pigs, and 13% (4/31) processed other animals (goat, deer (venison), wild boar). The results of the survey indicated that: a) the data on current methods used by the meat industry to microbiologically verify HACCP in meat plants at the time, could be used as an aid to develop a suitable practice method for HACCP verification for the purposes of Decision 2001/471/EC; and b) it would also assist the overall project in having major importance in developing and recommending alternative EU-equivalent method(s) for microbiological testing of red meats of proven acceptability to the industry; sampling plans for high- and low-throughput red meat operators; and hygiene performance criteria in the context of HACCP/QA. The results, however, also confirmed that at the time

the information was not available, either in the scientific literature at that time, or from current practice in meat plants, to allow decisions to be made on criteria for the microbiological monitoring in meat plants. For that, both experimental- and field-investigation-based work had to be carried out so to advance the understanding of the problem and to obtain necessary data.

To compare the performance (microbial recoveries) of the two EU-legislation-described carcass sampling methods (wet-dry swabbing and excision), bovine, ovine and porcine carcasses were individually inoculated by dipping in various suspensions comprising a marker organism (*Escherichia coli* K12 or *Ps. fluorescens*) alone or in combination with two meat-derived bacterial strains, and sampled using two standard methods: cotton wet-dry swabbing and excision. The samples were examined for Total Viable Count (TVC) and *Enterobacteriaceae* numbers (EC) using standard ISO methods. Overall, when all three meat species were taken together, average bacterial recoveries obtained by the swabbing expressed as a percentage of the appropriate recoveries achieved by the excision were 21% (range 2-100%) and 25% (range 9-47%) for TVC and *Enterobacteriaceae* (EC), respectively. Several factors potentially contributing to these relatively low, and highly variable, bacterial recoveries by swabbing were investigated in separate experiments. Neither the difference in size of the swabbed area (10, 50 or 100 cm^2 on beef carcasses) nor the difference in time of swabbing (20 or 60 minutes after inoculation of pig carcasses) had a significant effect on the swabbing recoveries of the marker organism used. In an experiment with swabs pre-inoculated with the marker organism and then used for carcass swabbing, on average 12% of total bacterial load was transferred inversely i.e. from the swab to the carcass during standard swabbing procedure. In another experiment, on average 14% of total bacterial load was not released from the swab into the diluent during standard swab homogenisation. Use of custom-made swabs with very abrasive buts, around which metal pieces of pan scourers were wound, markedly increased TVC recoveries from non-inoculated lamb carcasses at commercial abattoirs compared to cotton swabs. In spite of the observed inferiority of the cotton wet-dry swabbing method, as compared with the excision with respect to bacterial recovery, the former is

clearly preferred by the meat industry because it does not damage the carcass.

A large-scale evaluation of the two carcass sampling methods (wet-dry swabbing and excision) was undertaken in 23 commercial slaughterhouses with bovine, ovine and porcine carcasses to determine any influence of sampling method on carcass-derived counts of the natural microflora. Excision sampling produced significantly higher total aerobic and *Enterobacteriaceae* counts compared with those measured by wet-dry swabbing. Linear regression using transformed counts from near-adjacent carcases on processing lines for all three animal species revealed tenuous relationships between the swabbing- and excision-derived bacterial numbers. Thus it was not possible to calculate a factor, which allowed the inter-conversion of bacterial numbers for samples taken by each sampling method. Some factors, which confounded the identification of relationships between swabbing- and excision-based sampling, were investigated. Differences in bacterial populations were found on the surfaces of near-consecutive carcasses on the processing lines sampled. Uncertainty associated with laboratory analyses was also a contributing factor. The implications of these findings for HACCP-style process control verification were investigated by weekly carcass sampling at three commercial slaughterhouses over a 13-week period. In these plants, two sets of seven carcass samples were taken within a narrow time frame (< 1 hour). When the bacterial numbers from each of these sets of samples were compared, as much as a 4 Log CFU cm^{-2} difference in the total aerobic counts was observed. Thus it may not be appropriate to institute corrective actions based on a single week's statutory microbiological test results. The results of these findings are discussed in the context of slaughter process control and statutory hygiene enforcement.

1. Determination of the appropriateness of the HACCP plans in red meat abattoirs including the microbiological sampling programmes and criteria used (OBJECTIVE 1)

1.1 Introduction

An essential component of meat hygiene is avoiding the introduction of and multiplication of microbial pathogens on meat. Validated Hazard Analysis Critical Control Point (HACCP) plans that prevent contamination entering the system could provide the best assurance for food safety. The European Commission's Decision (2001/471/EC) requires the implementation of HACCP principles in fresh and poultry meat slaughterhouses, cutting plants and cold stores and introduces standard procedures for carrying out microbiological checks in fresh meat premises on cleaning and disinfection (for slaughterhouses and cutting plants) and on carcases (slaughterhouses only).

Verification is used to establish whether the HACCP plan is right for the actual operation of the abattoir and should show whether or not the monitoring and corrective actions are being properly applied. A good example of verification is the regular testing of carcases for the presence of microbial contamination. These microbiological test results provide a way of measuring how well the operator has controlled the slaughter and dressing procedure to avoid contamination. There are indications that the verification of HACCP using microbiological testing is only part of the verification and it is necessary for the FSA to be fully aware of activities in countries that are making major inputs to the development of HACCP based systems.

The information from this slaughterhouse survey and the overall project will be of major importance in developing and recommending alternative EU-equivalent method(s) for microbiological testing of red meats of proven acceptability to the industry; sampling plans for high- and low-throughput red meat operators; and hygiene performance criteria in the context of HACCP/QA.

1.2 Materials and Methods

A postal survey of slaughterhouses in England licensed under the Fresh Meat (Hygiene and Inspection) Regulations 1995 (As Amended) was conducted from 16 July to 16 August 2002. Quality Assurance departments of red meat abattoirs were asked about their HACCP systems in place, staff hygiene training, details on sampling plans, sampling analysis, and laboratory methods and reporting.

1.3 Results and discussion

The total response rate for the survey was 10% (31/299). This relatively low response rate may not be unexpected for a postal survey of the red meat plant community given the sensitive nature of HACCP verification and food hygiene as a subject matter.

Red Meat Plant Details

Approximately two-thirds of red meat plants that responded were full throughput plants (65%; 21/31), and 35% (11/31) were low throughput plants. The number of animals that full throughput plants processed per week ranged from 30 to 13,000 (mean = 4267 animals). The number of animals that low throughput plants processed per week ranged from 10 to 2,200 (mean = 331 animals). Over three-quarters of the meat plants processed cattle (77%; 24/31), 74% (23/31) processed sheep/lamb, a quarter (25%; 18/31) processed pigs, and 13% (4/31) processed other animals (goat, deer, wild boar).

Staff Training

Most full throughput meat plants claimed to employ between 50 and 500 staff (70%) whereas most low throughput plants employed less than 5 staff (82%). Full (70%) and low (64%) throughput meat plants claimed to have 81-100% staff employed that had received raw meat hygiene training. Most full (60%) and low (73%) throughput meat plants claimed to have some staff that had

received the basic meat safety certificate in raw meat training with full (70%) and low (36%) throughput meat plants claiming to have up to 20% staff employed that had received HACCP training (HACCP training of staff covered intermediate and advanced levels or other recognized HACCP courses).

Red Meat Plants with all seven HACCP principles in place

Most (85%; 17/20) of full throughput plants claimed they had all seven HACCP principles in place, whereas 73% (8/11) low throughput plants did not. The full HACCP system became operational in the 17 full throughput plants ranging from one to nine years ago (from 1993 to 2001), and is reviewed regularly in 16 out of these 17 (94%) full throughput plants. Most (94%; 16/17) of these full throughput plants carry out microbiological or hygiene monitoring in the plant.

The full HACCP system became operational in the 3 low throughput plants ranging from three to four years ago (from 1998 to 1999), and is reviewed regularly in one of these three (33%) low throughput plants. No microbiological or hygiene monitoring is carried out in these 3 low throughput plants

Red Meat Plants with some of the seven HACCP principles in place

Of the 3 full throughput plants that did not have all seven HACCP principles, 2 plants claimed to have some HACCP in place and 1 claimed to be planning to introduce a HACCP system within the next 12 months. Of the 8 low throughput plant that did not have all seven HACCP principles, 2 plants claimed to have some HACCP in place, and 6 did not. Four out of the 6 meat low throughput plants that did not have some HACCP in place claimed to be planning to introduce a HACCP system within the next 12 months, but 2 were not.

Microbiological or hygiene monitoring carried out in the red meat plant

Testing laboratory

Over half (56%; 9/16) of full throughput red meat plants used contract laboratories to examine samples, 5 (31%) used their in-house laboratory, 1 (6%) used both an in-house laboratory and a contract laboratory, and for the remainder (1; 6%) this was not recorded. Where both an in-house and contract laboratory were used to examine samples, samples for pathogen testing were examined by the contract laboratory.

Of the 17 testing laboratories, 15 (88%) were accredited for microbiological testing, 1 (6%) was not, and for 1 (6%) this information was not recorded. Over half of the laboratories that were accredited were accredited with UKAS (53%; 8/15), 4 (27%) with CLAS, and 3 (20%) with LABCRED. Of the 17 testing laboratories, 14 (82%) participated in a Quality Assurance (QA) scheme, 1 (6%) did not, and for 2 (12%) this information was not recorded.

Most of the laboratories that participated in a QA scheme used QM (43%; 6/14), 2 (14%) used EQA, 2 (14%) used both EQA and SMART QA (LabM), 1 (7%) used both EQA and FEPAS, 1 (7%) used both QM and FEPAS, 1 (7%) used both QM and SMART QA (LabM), and for 1 (7%) laboratory this information was not recorded.

The number of QA samples that laboratories claimed to test per year ranged from 2 to 93 samples. Over half (57%; 8/14) of the laboratories claimed to test QA samples on a monthly basis, 1 (7%) laboratory tested QA samples weekly to fortnightly, 1 (7%) every two months, 1 (7%) every six months, and for 3 (21%) laboratories this information was not recorded.

Pathogens were included as a target micro organism in QA samples tested by the majority (86%; 12/14) of the laboratories, of which 9 included *Salmonella* spp., 8 *Staphylococcus aureus*, 6 Escherichia coli O157, 5 *Campylobacter* spp., 4 *Yersinia enterocolitica*, and 2 included other pathogens (*Clostridium perfringens, Bacillus cereus, Listeria* spp.). Pathogens were not included as

a target micro organism in QA samples tested by one (7%) laboratory, and for one (7%) laboratory this information was not recorded.

Indicator organisms were included as target micro organisms in QA samples tested by the majority (93%; 13/14) of the laboratories, of which 13 included Total Viable Counts (TVCs), 12 *Enterobacteriaceae*, 12 coliforms, 9 *E. coli*, 4 *Pseudomonas* spp., and 1 included yeasts and moulds. Information on types of indicator organisms included as target micro organisms in QA samples tested by 1 (7%) laboratory was not recorded.

Under half (44%; 7/16) of the full throughput red meat plants employed examination methods for microbiological testing of samples taken at the plant that were all within the scope of accreditation, 4 (25%) used examination methods of which some were within the scope of accreditation, and 1 (6%) used examination method of which none were within the scope of accreditation. For 4 (25%) meat plants this information was not recorded.

Microbiological Testing of Carcases

Sixteen full throughput red meat plants carried out microbiological testing of carcases. The purpose of microbiological testing of carcases carried out by meat plants was as follows:

- Over half (56%; 9/16) of meat plants carried out testing to satisfy customer requirements, in-house quality control, and for statutory purposes.
- Two (13%) meat plants carried out testing to satisfy customer requirements and statutory purposes.
- One (6%) meat plant carried out testing to satisfy in-house quality control and statutory purposes.
- One (6%) meat plant carried out testing to satisfy in-house quality control.
- One (6%) meat plant carried out testing to satisfy customer requirements.

- For two (13%) meat plants this information was not recorded.

Carcass Sample Sites and Methods

- Two-thirds (63%; 10/16) of the full throughput red meat plants that carried out microbiological testing of carcases sampled cattle carcases; half (50%; 8/16) sampled sheep/lamb carcases and 7 plants (44%) sampled pig carcases. There were considerable variations in sampling methods used between plants and animal species. This is summarised in Table 1 & 2.

Table 1. Sampling methods used

	Wet/dry swabs	Wet swab	Excision	Other
cattle	50%	10%	10%	30% mixture of wet & dry & excision
sheep	50%	12.5%	25%	12.5% used post pack sample
pigs	86%	0	14%	

Table 2. Area sampled

	10 cm^2	20 cm^2	25 cm^2	50 cm^2	100 cm^2	Other
cattle	10%	10%	10%	0	40%	30%
sheep	12.5%	12.5%	12.5%	0	12.5%	50%
pigs	29%	25%	0	29%	29%	

(A sampling area of 5cm^2 was also reported and two plants also reported used large areas of the carcass)

For cattle carcases. Sites sampled on included brisket (70%; 7/10), flank (70%; 7/10), neck (70%; 7/10), rump (70%; 7/10), sirloin (20%; 2/10), and other sites (20%; 2/10, forequarter shin, hindquarter shin). Two meat plants did not record which sites they sampled and 1 meat plant recorded that it sampled ‘various’ sites. The number of carcases claimed to be sampled per week are 1 (10%; 1/10), 5 (60%; 6/10), and 10 (10%; 1/10). Two meat plants did not record the frequency of sampling.

Half of the meat plants sampled cattle carcases at the end of line (50%; 5/10), 3 (30%) at the pre-wash, 1 (10%) at both the pre-wash and post-chiller, and 1 (10%) at post pack. Carcases sampled at post chiller were claimed to be sampled in less than 12 hours after chilling.

For sheep carcases. Sites sampled on included breast (75%; 6/8), flank (75%; 6/8), neck (50%; 4/8), crutch (38%; 3/8), and other sites (50%; 4/8, hind limb (rump), loin, leg, brisket, lateral thorax). One meat plant did not record which sites it sampled and one meat plant recorded that it sampled 'various' sites. The number of carcases claimed to be sampled per week are 1 (12%; 1/8), 5 (62%; 5/8), and 10 (12%; 1/8). One meat plant did not record the frequency of sampling sheep/lamb carcases.

Over a third of meat plants sampled carcases at the end of line (38%; 3/8), 2 (25%) at the post chiller, 1 (12%) at the pre-wash, 1 (12%) at both the pre-wash and post-chiller, and 1 (10%) at post pack. Carcases sampled at post chiller were claimed to be sampled in less than 12 hours after chilling.

For pig carcases. Sites sampled included belly (86%; 6/7), hind limb (86%; 6/7), mid back (86%; 6/7), cheek (71%; 5/7), and shoulder (14%; 1/7). One meat plant did not record which sites it sampled. The number of carcases claimed to be sampled per week are 5 (71%; 5/7) and 20 (14%; 1/7) and 1 meat plant did not record the frequency of sampling.

Two meat plants sampled pig carcases at the end of line (29%; 2/7), 2 (29%) at the pre wash, 1 (14%) at the post wash, 1 (14%) at the post chiller, and 1 (14%) at both the pre-wash and post-chiller. Carcases sampled at post chiller were claimed to be sampled between 12 and less than 24 hours after chilling.

<u>Other species</u>

Six percent (1/16) of the full throughput red meat plants that carried out microbiological testing of carcases sampled other animal species carcases

(i.e. deer). It recorded that it sampled 'various' sites on 1 carcase per week but sampled deer carcases at post pack sampling a whole cut (post pack sample).

Microbiological checks of surfaces

Sixteen full throughput red meat plants carried out microbiological testing of surfaces (e.g. machine or equipment surfaces). The purpose of microbiological testing of environmental areas carried out by meat plants was as follows:

- Almost two-thirds (62%; 10/16) of meat plants carried out testing to satisfy customer requirements, in-house quality control, and for statutory purposes.
- One (6%) meat plant carried out testing to satisfy customer requirements and statutory purposes.
- One (6%) meat plant carried out testing to satisfy in-house quality control and statutory purposes.
- Two (13%) meat plants carried out testing to satisfy in-house quality control.
- One (6%) meat plant carried out testing to satisfy customer requirements.
- For one (6%) meat plant this information was not recorded.

Thirty-eight percent (6/16) of meat plants tested between 10 to 20 environmental samples within a two week period, 5 plants (31%) tested 30 or more environmental samples, 4 plants (25%) tested between 20 to 30 samples, and 1 plant (6%) tested less than 10 environmental samples. Meat plant areas and/or equipment tested included the following:

- Abattoir equipment
- All equipment and surfaces in abattoir
- All meat contact surfaces (e.g. butchers' blocks, conveyors, saws, bandsaw, tables, skinners, trays, knives, gambrels,

tenderiser, offal racks, chute, rodding tool, steels, belts, scales, meat bowl, slicing machine, gloves (chain-mail) etc.)

- Walls
- Slaughter hall
- Boning halls
- CPM, CPM-offal

Plant environment

Most (88%; 14/16) meat plants claimed to test the plant environment. Over one-third (36%; 5/14) of meat plants took 10 environmental samples per week, 3 plants took over 15 samples per week (15 (1/16), 25 (1/16), 30 (1/16)), and 3 plants took five or less samples per week (1 (1/16), 2 (1/16), 4 (1/16), 5(1/16)). For 2 meat plants this information was not recorded.

Half of the meat plants sampled the plant environment using the surface swab method (50%; 7/14), 3 (21%) used the wet/dry swab method, 2 (14%) used both the surface swab and wet/dry swab methods, 1 (7%) used the prepared agar slide method, and 1 (7%) used the petri-film method.

Forty-three percent (6/14) of meat plants used an area size of 10 cm^2 for sampling the plant environment, 3 (21%) used 20 cm^2, 2 (14%) used 25 cm^2, 2 (14%) used 100 cm^2, and 1 (7%) used 15 cm^2.

Plant equipment

All (100%; 16/16) meat plants claimed to test the plant equipment. Almost two-thirds (63%; 5/16) took 10 samples per week, six plants took between 10 and 30 samples per week (11 (3), 25 (2), 30 (1)), 3 plants took ≤ 5 samples per week (3 (1), 5 (2)), and 1 plant took 168 samples per week. For 1 meat plant this information was not recorded.

Half of the meat plants sampled the plant equipment using the surface swab method (50%; 8/16), 3 (19%) used the wet/dry swab method, 2 (13%) used

both the surface swab and wet/dry swab methods, 1 (6%) used the pre-prepared agar slide method, 1 (6%) used the petri-film method, and 1 (6%) used both the wet/dry swab and ATP swab methods.

Thirty-eight percent (6/16) meat plants used an area size of 10 cm^2 for sampling the plant equipment, 4 (25%) used 20 cm^2, 3 (19%) used 100 cm^2, 2 (13%) used 25 cm^2, and 1 (6%) used 15 cm^2.

Process water

Most (88%; 14/16) meat plants claimed to test the plant process water. Meat plants took between 1 to 10 process water samples per week or between 1 to 5 samples per month. Over half (56%; 9/16) of the meat plants sampled the process water using a sample bottle, and one (6%) used a dipper. For four (25%) meat plants this information was not recorded.

Thirty-eight percent (6/16) meat plants used a volume size of 10 cm^3 for sampling the process water, 2 (13%) used 500 cm^3, and 1 (6%) used 50 cm^3. For 5 (31%) meat plants this information was not recorded.

Comments from slaughterhouse operators on sampling carcases and environmental sites

- It was considered more beneficial to test product ‘as sold’ to customer than from the carcass. The carcass would only be swabbed if results were continually high.
- Taking swabs in the environment of the chiller is a difficult task without accidentally contaminating swabs. It’s impossible to take at the end of the line because of space and time restrictions. In the chiller it’s a crowded environment without anywhere to store sampling equipment and paperwork.
- The levels set by legislation seem low compared with industry standard figures.

- Water is not actually used in processing but water outlets (taps/hoses) in production areas are tested monthly as per statutory requirements
- Place emphasis on food contact surfaces and equipment rather than monitoring the bacterial levels of surfaces the food will not touch. We retest all samples deemed to be unacceptable by the HACCP Regulations 2002 with regard to environmental swabbing.
- In process of adjusting customer and new legislation requirements into microbiology analysis programme.

Sample transport and storage details

- Three-quarters (75%; 12/16) of meat plants claimed to transport samples to the laboratory at below 4°C, and 3 (19%) transported samples at or above 4°C. For 1 meat plant this information was not recorded.
- Nearly two-thirds (63%; 10/16) of meat plants claimed to transport samples to the laboratory using a cool box, 2 plants (13%) used a mobile refrigerator, 2 (13%) used a refrigerated vehicle, and 2 (13%) used no temperature controlled transportation method, i.e. delivered to the laboratory 'by hand'.
- Nearly two-thirds (63%; 10/16) meat plants claimed to transport samples to the laboratory within 2 hours, 3 plants (19%) between 2 and 4 hours, and 3 plants (19%) between 4 and 8 hours.
- Most (94%; 15/16) samples are stored at the laboratory at below 4°C, 1 (6%) meat plant reported that samples submitted to the laboratory were stored at 4°C.
- Meat plants reported the time that samples were processed after arrival at the laboratory was:
    - within 2 hours (44%; 7/16)
    - between 2 and <4 hours (31%; 5/16)
    - between 4 and <8 hours (6%; 1/16)
    - between 8 and <24 hours (13%; 2/16)
    - not known (6%; 1/16)

Microbiological Sampling Analysis Details:

Cattle. Ten full throughput red meat plants carried out microbiological testing of cattle carcases and carried out the following colony counts on cattle carcases:

- All 10 (100%) meat plants tested for TVCs. Seven used the TVC test with an incubation temperature of 30°C, 1 an incubation temperature of 37°C and for 2 meat plants the TVC incubation temperature was not recorded.
- Six (60%) plants tested for *Enterobacteriaceae.*
- Five (50%) plants tested for coliforms and 5 (50%) tested for *E. coli.*
- Four (40%) plants tested for *Staph. aureus.*
- None tested for *Pseudomonas* spp.

Seven (70%) plants tested cattle carcases for pathogens, while 3 (30%) meat plants did not. Seven (70%) meat plants tested for *E. coli* O157, 3 (30%) meat plants tested for *Salmonella* spp but none tested for *Campylobacter* spp.

Sheep/Lamb. Eight full throughput red meat plants carried out microbiological testing of sheep/lamb carcases. The meat plants carried out the following colony counts on sheep/lamb carcases:

- All eight (100%) meat plants tested for TVCs. Five used the TVC test with an incubation temperature of 30°C, 1 used an incubation temperature of 22°C and for 2 meat plants the TVC incubation temperature was not recorded.
- Five (63%) plants tested for *Enterobacteriaceae.*
- Five (63%) plants tested for coliforms and for *E. coli.*
- Five (63%) plants tested for *Staph. aureus.*
- None tested for *Pseudomonas* spp.

Five (63%) plants tested sheep/lamb carcases for pathogens, while 3 (37%) meat plants did not. Four (50%) meat plants tested for *E. coli* O157, 4 (50%) for *Salmonella* spp but none tested for *Campylobacter* spp.

Pigs. Seven full throughput red meat plants carried out microbiological testing of pig carcases. The meat plants carried out the following colony counts on pig carcases:

- All seven (100%) meat plants tested for TVCs. Three used the TVC test with an incubation temperature of 30°C, 2 37°C and 1 with an incubation temperature of 22°C and 37°C For 1 plant the TVC incubation temperature was not recorded.
- Six (86%) plants tested for *Enterobacteriaceae.*
- 1 (14%) plant tested for coliforms for *E. coli.*
- 1 (14%) plant tested for *Staph. aureus.*
- 1 (14%) plant tested for *Pseudomonas* spp.

Three (43%) plants tested pig carcases for pathogens, while 4 (57%) meat plants did not. One meat plant tested for *E. coli* O157, 3 (43%) for *Salmonella* spp. none tested for *Campylobacter* spp. nor for *Y. enterocolitica*

Other animal species (i.e. venison). One full throughput red meat plant carried out microbiological testing of deer carcases carrying out TVC at an incubation temperature of 30°C. Coliforms, *E. coli, Enterobacteriaceae*, S*taph. aureus, Pseudomonas* spp. were not tested. *E. coli* O157 was tested but other pathogens (*Salmonella* spp. *Campylobacter* spp., *Y. enterocolitica*) were not.

Environmental samples

Sixteen full throughput red meat plants carried out microbiological testing of environmental samples with the following colony counts on environmental samples:

All 16 (100%) meat plants tested for TVCs, 8 (50%) used the incubation temperature of 30°C, 3 (19%) a temperature of 37°C, 1 (6%) 32°C, 1 (6%) 22°C and 37°C and for 3 (19%) meat plants the TVC incubation temperature was not recorded.

- Three (19%) plants tested for *Enterobacteriaceae.*
- Eight (50%) plants tested for coliforms.
- One (6%) plant tested for *E. coli.*
- None tested for *Staph. aureus.*
- None tested for *Pseudomonas* spp.
- One plant (6%) tested for another organism, i.e. *Clostridium estertheticum.*

Four (25%) plants tested for pathogens, while 12 (75%) meat plants did not.

- Two (12%) meat plants tested for *E. coli* O157
- One (6%) meat plant tested for *Salmonella* spp.
- None tested for *Campylobacter* spp. or for *Y. enterocolitica*
- For 1 (6%) meat plant the pathogen type tested was not recorded

<u>Additional comments from slaughterhouse operators on microbiological tests carried out on carcass and environmental samples</u>

- The test for TVC's at 30°C for 72 hours is UKAS accredited, however the Meat (HACCP) Regulations 2002 requests that TVC at 37°C for 24 hours is carried out. This is recorded as non-UKAS accredited on the final report.
- Recently stopped testing for coliforms as had negative results. This is also not a customer or legal requirement.

<u>Laboratory methods/Techniques Details:</u>

Animal species (cattle, sheep, pig, other species)

- Most meat plants used laboratories that did not pool samples prior to microbiological examination (60%; 10/16), 5 (31%) used laboratories that did, and for 1 meat plant this information was not recorded.
- Most (88%; 14/16) meat plants did not use ATP bioluminescence to assess hygiene, 1 (6%) did, and for 1 meat plant this information was not recorded.

- Most of the tests/microbiological methods (colony counts, pathogens) carried out were conventional culture methods.
- Most of the microbiological methods carried out were based on ISO/BS Standards.
- Most results from Colony Count tests were reported as cfu/gram or cfu/cm^2
- Most results from pathogen tests were reported as per 25 g (29%; 8/28).

Environmental samples

- Most (88%; 14/16) meat plants did not use ATP bioluminescence to assess hygiene, 1 (6%) did, and for 1 meat plant this information was not recorded.
- Most of the tests/microbiological methods (colony counts, pathogens) were carried out using conventional culture methods.
- Most of the microbiological methods were carried out were based on ISO/BS Standards.
- Most results from Colony Count tests were reported as cfu/gram or cfu/cm^2
- Most results from pathogen tests were reported as per 25 g (29%; 8/28).

Additional comments from slaughterhouse operators on sample analysis details

- Protein detection is not ATP based but is used as a rapid check to enable a re-clean to be performed immediately. Protein detection used 10 swabs per day.
- Microbiological analysis (methods, frequency, reporting) has been changed to comply with the new legislation.

Microbiological results and assessment of hygiene standards in the plant

Most (81%: 13/16) of meat plants that carried out microbiological testing used these results to assess hygiene standards in the plant.

1.4 Concluding remarks

The data collected in the survey, on current methods used by the meat industry to microbiologically verify HACCP in meat plants, can be used as an aid to develop a suitable practice method for HACCP verification for the purposes of Decision 2001/471/EC.

It will also assist the overall project in having major importance in developing and recommending alternative EU-equivalent method(s) for microbiological testing of red meats of proven acceptability to the industry; sampling plans for high- and low-throughput red meat operators; and hygiene performance criteria in the context of HACCP/QA.

This survey however confirmed that the information was not available, either in the scientific literature at that time, or from current practice in meat plants, to allow decisions to be made on criteria for the microbiological monitoring in meat plants.

1.5 References

European Commission (EC). Proposals for Regulations on the Hygiene of Foodstuffs. COM (2000) 438 final. Brussels, 14.07.2000.

2. Experimental determination of the performance of carcass sampling/microbiological methods (OBJECTIVES 2 & 3)

2.1 Introduction

Traditional physical meat inspection in slaughterhouses is based on meat inspection procedures developed in the 19th Century. Visual inspection does not identify pathogenic microorganisms such as *Salmonella, Campylobacter, E. coli* O157:H7 or *Yersinia* (*7, 10, 11, 26*). To improve the control of such pathogens via improvements in process hygiene, the use of a Hazard Analysis Critical Control Points (HACCP) system has been promoted and implemented in the EU (*2, 3, 16*). In the UK, the Government/Industry Working group on Meat Hygiene (*2*) supported the incorporation of HACCP principles into the legislation governing slaughterhouses. Subsequently, a recent EU Commission Decision (2001/417/EC) required that fresh red meat operators must have validated HACCP-based systems in place and conduct regular checks to determine process hygiene trends. The stipulated process control checks involve the sampling and microbiological testing of carcasses.

Many techniques have been developed in the past for enumerating microorganisms on meat surfaces (*18*). The main considerations in method selection for microbiological testing of foods include turn around time, cost per test, capital for equipment, analyst training, facility layout and customer requirements (*24*). However, the EU Commission Decision (2001/471/EC) specifies only a single carcass-sampling technique: excision. There is provision, however, for other methods such as wet/dry swabbing to be used as long as they have been properly validated.

There is general agreement that excision is the most effective bacterial carcass sampling method (*1, 6, 22, 23, 25*) and thus it represents the reference method against which other sampling methods should be evaluated.

Nevertheless, it is often stressed, including by the meat industry, that excision is unacceptable or impractical as it results in visible evidence of sampling on

the carcass, which may reduce its commercial value (*17*). Although swabbing recovers only a proportion of microbial load present on carcass surface, some authors consider its performance as acceptable as long as reliable and trained staff perform it (*17, 25*). In addition, the diluent fluid from swab samples can be plated directly by a variety of surface and pouring plating techniques. In contrast, excision samples require further processing stages such as homogenisation and filtration before plating making the laboratory analyses of excision samples more time consuming and costly. For these reasons, it has been claimed that, within a given period of time, higher numbers of swab samples can be examined compared with excised samples (*25*). It was suggested (*25*) that, because a larger number of samples can be examined by swabbing compared with excision, the former method has advantages in overcoming problems caused by significant variability of bacterial numbers on carcasses at the same abattoir. Furthermore, there are views (*17*) that swabbing enables sampling of a wider area on the carcases, which may improve the detection of infrequent pathogens such as *E. coli* O157 or *Campylobacter*. In other words, sampling of an increased carcass area by swabbing may be particularly advantageous when the principal aim is to determine pathogens prevalence, rather than exact microbial counts per surface area (*17*).

One of the acknowledged problems with swabbing, however, is the high variability of recoveries achieved, ranging between 0.01% and 89% of those achieved by excision (*8, 9, 12*). Swabbing variability is not species-specific, and has been observed between different carcasses of the same species and between different areas of a single carcass (*12*). In addition, numerous swab material-, staff-, and time-related factors can contribute to the variation associated with swabbing (*4, 20, 21*). The combination of these factors can make interpretation of process control trends, and the HACCP-based process control verification based on them, difficult. Furthermore, these effects make comparisons between different abattoirs and between different published studies very difficult. There are views (*19*) that red meat carcass swabbing is not a useful method for practical process control at present, because the

extent of its variability and underlying mechanisms are not sufficiently understood (*19*).

Nevertheless, the wet-dry carcass swabbing is now the most commonly used carcass-sampling method in the context of mandatory HACCP-based systems in red meat abattoirs within the EU. However, a large-scale evaluation of swabbing method is still necessary, in order to unequivocally assess its suitability for the HACCP verification purposes. In this initial study, the performance of a carcass swabbing method was evaluated under controlled experimental conditions using artificial contaminants. A subsequent study (Chapter 3) has evaluated the wet-dry carcass swabbing method performance under commercial processing conditions.

2.2 Materials and methods

Bacterial inocula

The bacterial strains used for the inoculation experiments were from the collection of the School of Veterinary Science at The University of Bristol: marker 1 (*Escherichia coli* K12; resistant to 200 ppm nalidixic acid), marker 2 (*Pseudomonas fluorescens; resistant to* 100 μg ml^{-1} rifamapicin and 10 ppm nalidixic acid), and two strains isolated from pig carcasses (*Staphylococcus* spp. and *Micrococcus* spp.). The organisms were grown in 1000 ml volumes of Heart Infusion Broth (HIB, Difco, Sparks, MD, USA) at 37˚C for 24 h.

Carcass inoculation

Large plastic containers of sufficient volume to contain a beef carcass side were half-filled with a known amount of tap water preheated to 41°C. Pre-calculated volumes of cultures of the marker 1 bacteria and the two meat strains (separately or combined, depending on the experiment; see below) were added to the water and stirred to achieve bacterial concentration of

approximately 5 Log CFU ml^{-1}. Using a winch, a dressed beef carcass side or whole dressed porcine or ovine carcass was individually lowered and entirely submerged into the bacterial suspension. Bacterial suspensions were freshly prepared for each carcass/carcass side. The temperature of the suspension immediately after carcase immersion was typically between 37 and 39°C. After five minutes, the carcass was lifted from the suspension and left hanging to drain, depending on experiment, for 5, 20 or 60 minutes before sampling.

Experimental design

The study was carried out as five separate experiments.

Experiment A: The aim was to compare total bacteria and *Enterobacteriaceae* recoveries from inoculated carcasses sampled by swabbing against the excision method. Each of the 18 bovine, 18 ovine and 18 porcine carcasses were uniformly inoculated with a cocktail comprising the marker 1 and the two meat-derived strains as described above, before taking 6 excision samples and the same number of swab samples from each carcass. Samples were taken from different, randomly chosen sites on each carcass using the sampling protocols described below. Total aerobic plate counts (TVC) and *Enterobacteriaceae* numbers were determined from the samples using the standard microbiological methods described below.

Experiment B: The aim was to evaluate whether the area sampled using the swabbing method influenced efficiency of bacterial recovery from the carcass surface. Twelve bovine carcasses were inoculated only with bacterial marker 1 as described above. Excision and swabbing were used to collect samples as described for experiment A. The areas used for swab sampling were 10 cm^2, 50 cm^2 and 100 cm^2.

Experiment C: The aim was to evaluate whether the length of time that marker bacteria were on the carcase surface influenced surface attachment and consequently bacterial recoveries using the swabbing method. Porcine carcasses were used because skin has a more uniform composition than that

of bovine or ovine carcasses whose surfaces are non-uniformly composed of fatty tissue, membrane and muscle. Twelve porcine carcasses were inoculated only with the marker 1 using the technique described above. Carcases were initially sampled using the swabbing method before sampling using excision from the previously swabbed site. To mimic different processing line speeds, samples were collected 20 minutes and 60 minutes after carcass inoculation. These two times were selected to reflect the shortest and the longest expected carcass processing time typically encountered at a commercial abattoir. In addition, at the laboratory, swabs were either homogenised by vortexing once before plating (according to the standard method) or transferred to fresh diluent and subjected to second vortexing in the fresh diluent, before plating. The counts of marker 1 were determined as described below. A secondary aim of this experiment was to determine the proportion of microflora not extracted from the swab during a standard laboratory analyses.

Experiment D: The aim was to evaluate whether reverse transfer of bacteria from the swab back to the carcass occurs during repeated swabbing strokes. Swabs were individually submerged into a suspension of the marker 2 (described above), left to drain for 5 minutes, and then used for swab sampling of 12 non-inoculated porcine carcasses. Subsequently, the swabbed area was sampled by excision. Then, the marker was determined on the swab used for the swabbing, as well as on the excised sample from the swabbed area.

Experiment E: The aim was to determine whether/how much the abrasiveness of the material used for swabbing influenced bacterial recoveries. Carcass samples (12 bovine, 30 ovine and 12 porcine) were taken at a commercial abattoir during routine processing using: a) the standard cotton swabs that are described below for the standard wet-dry swab method, and b) standard swabs but with sterilised spiral metal pieces taken from pan scourers ("Spontex Tough Scourers", Mapa Spontex U.K. Ltd) aseptically wound around their cotton buds ("metallic" swabs). The sampling procedures were the same as described below.

Excision sampling method

Excision-based sampling involved removing a sliver of tissue of 5 cm^2 area and 2 mm thickness from four sites on each carcass sampled. A flame-sterilised stainless steel borer (2.52 cm diameter) was firmly pressed into the carcass surface and twisted to make an initial cut to a depth of ~3 mm. A sterile disposable scalpel (Swann Morton, Sheffield, England) and forceps (Scientific Lab Supplies, Nottingham England) were used to cut the tissue sliver free from the carcass. Each sample was stored in a single 304 mm x 177 mm sealed polythene stomacher bag (Seward, Thetford, England) on crushed ice until microbiological analyses.

Wet-dry swabbing sampling method

Jumbo head cotton swabs (Sterilab Services, Harrogate, England) were used for wet-dry swab sampling. Samples were collected within an area of either 100 cm^2 (bovines) or 50 cm^2 (ovines and porcines) unless specified differently. The sampling area was delineated by an alcohol-sanitised stainless steel template. For each sampling area, a swab was moistened in maximum recovery diluent (MRD; Bacteriological Peptone [Oxoid, Oxford, UK; L37] 1 g, sodium chloride 8.5 g in 1000 ml), and then rubbed firmly across the carcass surface using ten strokes in each of the horizontal, vertical and diagonal directions. Swabs were rolled between the thumb and index finger as they were rubbed across the carcass surface. Immediately after rubbing with the MRD-moistened swab, the procedure was repeated at the same site using a dry swab. Each pair of wet and dry swabs was combined into a single sample in a sterile universal containing 20 ml MRD and stored on crushed ice until microbiological analyses.

Microbiological Analyses Methods

TVC and *Enterobacteriaceae* numbers were determined by standard plate count methods (*11* and *12*, respectively). Briefly, microbiological analyses involved the addition of 100 ml of peptone saline [10 g protease peptone, 5 g NaCl, 9 g $Na_2HPO_4.12H_2O$, 1.5 g KH_2PO_4, to 1000 ml] to each stomacher bag containing an excised set of samples, followed by homogenisation for 1 minute using a stomacher (Model number BA 6021, Seward, UK). Individual wet-dry swab samples were weighed and their volume adjusted to 25 ml with MRD. The tubes containing the swab samples were vigorously vortexed (Model SGP 202 O1OJ Fisons, Ipswich, UK) for 1 minute. Each sample homogenate (excision or swab) was then diluted decimally in peptone saline and 1 ml aliquots added to appropriately labelled Petri dishes. For TVC, 15 ml of tempered (46°C) PCA medium (Oxoid CM325) was added to each Petri dish, mixed and allowed to solidify. For *Enterobacteriaceae*, 15 ml of tempered (46°C) VRBGA medium (Oxoid CM485) was added, mixed and allowed to solidify. TVC were incubated at 30°C for 72 h before colonies were counted. *Enterobacteriaceae* were incubated at 37°C for 24 h. Confirmation of *Enterobacteriaceae* was done by testing for oxidase and the ability to metabolise glucose. Bacterial numbers on decimally diluted plates were converted into CFU cm^{-1} in a standard manner (*10*).

Counts of *E. coli* K12 were determined in the samples by plating appropriate decimal dilutions onto MacConkey agar (N° 3; Oxoid) supplemented with 200µg ml^{-1} nalidixic acid (Oxoid) and incubation at 37°C for 24 h.

Counts of *Ps. fluorescens* were determined by plating on *Pseudomonas* agar (Oxoid) containing glycerol (10g l^{-1}; Sigma), pre-weighted CFC supplement (Cetrimide, Fucidin and Cephalosporin; Oxoid) and 10 µg ml^{-1} nalidixic acid, after incubation at 30°C for 24-48 h.

Analysis of results

The results were calculated (mean values, standard deviation, differences between means) using SPSS software.

2.3 Results and Discussion

Overall, when the results obtained by swabbing from the three meat species used were combined, then expressed as a percentage of the appropriate excised samples, the average recoveries for TVC and *Enterobacteriaceae* (EC) respectively were 21% and 25% (Table 1). Although these average values are roughly equivalent, the range of recoveries varied markedly. TVC recoveries ranged between 2% and 100% whereas the range observed for EC was narrower at between 9% and 47%. Due to large variability of the results, differences in bacterial recoveries between animal species were not significant. The descending trend of TVC recoveries was bovine>ovine>porcine, whilst of EC was bovine>porcine>ovine (Table 1). High between-species variabilities in swabbing performance was also observed in early studies (*12*), but with different descending order of recoveries: porcine>ovine>bovine. Also, more recently, the performance of the carcass swabbing method was shown to vary with animal species with better recoveries shown from beef carcases when compared with pork (*9*). Whilst in the latter two studies differences in animal species-related natural microflora and the processing environments may have caused different recoveries, in the present study the role of such factors was minimised by the carcass dip-inoculation with a standardised microflora.

Even within the same animal species, large variations in TVC and/or EC recoveries were observed (note high SD values in Table 1). This was in spite of the fact that the inoculated carcasses had as uniform distribution of microflora as was practically achievable in an abattoir experiment. This observation suggests that other factors, in addition to animal species and type of microflora, influence results obtained by swabbing. Generally, numerous older studies agree that cotton swabbing recovers only a portion (<1%-89%)

of carcass microflora recoverable by excision. Other studies have shown that sponge-swabbing also recovers significantly lower TVC than excision (*5, 6*). However, some more recent studies cast some doubts as to whether average bacterial recoveries achieved by swabbing and excision methods are significantly different. In a study involving four beef and two pig abattoirs (*9*), TVC recoveries: a) were not significantly different between swabbing and excision methods at three beef abattoirs, b) were significantly lower by swabbing than by excision at fourth beef abattoir and at both pig abattoirs. Therefore, it is important to keep in mind that the existence or not of significant differences between bacterial recoveries by the two methods is dependant on a number of factors including the type of the swab used, whether the swabbed surface is fat or lean, whether it is skin (e.g. pork) or meat (e.g. beef), or whether the samples were collected immediately after processing or after a period of cold storage (*9, 20, 27*). Consequently, it is very difficult to compare performance of swabbing methods between different studies. In the present study, the relevance of some factors for bacterial recoveries by the swabbing method was evaluated.

Table 1. TVC and *Enterobacteriaceae* recoveries from inoculated carcasses* by the swabbing method as compared to those by the excision method (*Experiment A*)

Animal species	**Total viable bacteria counts** log cfu/cm^2±SD (% of excision)		***Enterobacteriaceae* counts** log cfu/cm^2±SD (% of excision)	
	By excision	By swabbing	By excision	By swabbing
Bovine (n=18)	4.8±1.3	4.9±0.5 (100%)	5.0±0.9	4.7±0.9 (47%)
Ovine (n=18)	5.1±1.2	4.4±0.4 (23%)	3.9±0.9	2.9±1.2 (9%)
Porcine (n=18)	5.6±0.2	3.8±1.0 (2%)	3.1±0.9	2.6±1.1 (32%)
Overall (n=18)	5.1±1.1	4.5±0.2 (21%)	3.9±0.9	3.4±1.1 (25%)

* Carcasses inoculated by a cocktail of *E. coli* (K12) and two bacterial strains previously isolated from respective meat species

The results presented in Table 2 show microbial recoveries obtained by sampling carcass areas of different sizes. Although the recoveries by swabbing slightly increased as the sampled area increased, the differences were not significant. Nevertheless, it could be assumed that sampling of larger carcass areas would have practical advantages, as it is more representative of the whole carcass surface and may diminish the effects of non-uniform distribution of microflora.

Table 2. Effects of sampled area on the marker organism (*E. coli* K12) recoveries from inoculated beef carcasses by the swabbing method (*Experiment B*)

Sampling method	Recovery of the marker organism log cfu/cm^2 $\pm$ SD (% of total)		
	Sampled 10 cm^2 (n=12)	Sampled 50 cm^2 (n=12)	Sampled 100 cm^2 (n=12)
1. By swabbing	3.7 ± 0.2 (63%)	3.3 ± 0.4 (66%)	3.4 ± 0.4 (70%)
2. By excision from the swabbed area	3.3 ± 0.5 (37%)	2.2 ± 1.5 (34%)	2.6 ± 1.3 (30%)
3. Swabbing + excision (1+2)	3.9 ± 0.3 (100%)	3.4 ± 0.9 (100%)	3.4 ± 0.9 (100%)

With respect to time duration between the carcass dressing and the microbiological sampling, significant variations could be expected between different abattoirs operating at different line speeds. Published studies indicate that irreversible attachment of bacterial cells to the carcass surface can occur within time period varying between 30 min and several hours (*4*), which inversely influence bacterial recovery by swabbing. To determine whether this factor has a relevance under normal abattoir practice, carcass samples were taken 20 min (reflecting fast processing line) and 60 min (reflecting slow processing line) after carcass inoculation (Table 3). No significant differences in bacterial recoveries by swabbing within the timeframe applied were found in

this study (Table 3). Nevertheless, these results were obtained with pork (i.e. skin-on) carcasses only, and do not necessarily allow extrapolations to skin-off carcasses such as beef and lamb.

Table 3. Effects of some bacterial attachment-related factors on recoveries by swabbing (*Experiments C and D*)

Factors evaluated (N^{o} of samples)	Recovery of respective marker organism as percent of total recovery*		
	Lowest	Highest	Average
Experiment C: marker 1** inoculated on the porcine carcass but not on the swab			
Swabbing 20 min after carcass inoculation (n=12)	17%	27%	24%
Swabbing 60 min after carcass inoculation (n=12)	12%	30%	22%
Experiment D: marker 2*** inoculated on the swab but not on the porcine carcass			
Marker 2 remaining on the pre-inoculated swab after carcass swabbing (n=12)	77%	96%	88%
Marker 2 transferred from the pre-inoculated swab onto swabbed carcass (n=12)	23%	4%	12%

*Total recovery of the respective marker = recovery from the swab + recovery from the excised swabbed area; ** *E. coli* K12; *** *Ps. fluorescens*

Results of published studies obtained with non-meat surfaces indicated that some swab-related factors could significantly affect bacterial recoveries (*20*). In particular, it was found that the attachment of bacteria onto the swab material itself could enhance bacterial transfer from the sampled surface onto the swab (*20*). In the present study, the extent of bacterial transfer in an undesirable direction i.e. from cotton swab onto the carcass surface was investigated by using swabs pre-inoculated with the marker bacteria (Table 3). During the standard swabbing strokes, on average 12% of the marker's load was actually transferred swab-to-carcass and would not be accounted for by the standard swabbing procedure. On the other hand, the intensity of bacterial attachment to the swab material can also influence intended release of bacteria from the swab into the diluent during the swab homogenisation phase of analyses. In the present study, when swabs were once-homogenised

(vortexed) in diluent in an usual manner and then removed and transferred into a small volume of fresh diluent and re-vortexed, it was found (results not shown) that an additional portion (14% of the total recovery) of the marker organism could be recovered from the second homogenate. This suggests that a portion of bacterial load, not released from the swab, is not accounted for during the standard microbiological procedures. Taking into account together the swab-to-carcass transfer of bacteria and the bacterial portion attached to, and not released during vortexing from the swab material, it appears that a proportion of the carcass microflora not-accounted for by the swabbing could reach one-quarter (on average, but with marked variability) of the actual load.

There is little doubt that important requirements for any material to be used for constructing swabs include: a) sufficient abrasiveness to detach bacteria from the carcass surface, and b) ability to readily release the bacteria during homogenisation. It was hypothesised that metal would reasonably satisfy both requirements, and so custom-fabricated “metallic” swabs were used for swab-sampling of non-inoculated carcasses under commercial processing conditions. TVC recoveries by “metallic” swabs markedly increased when compared with those obtained using standard cotton swabs (Table 4). The observation was conserved across all three species that were sampled. However, due to large between-carcass variations, the increased average recovery by “metallic” swabs was statistically significant only with lamb carcasses. Nevertheless, further developmental research aimed at more refined, highly abrasive i.e. “scouring-type”, metal material-based swabs appear to be very promising.

Table 4: The effect of swab abrasiveness on bacterial recovery by swabbing (*Experiment E*)

Carcass samples (No)	TVC recovery Mean log cfu/cm^2 (min-max)	
	Abrasive ("metallic") swab	Cotton wet/dry swab
Sheep (n=30)	4.3 [a] (3.5-5.2)	3.9 [b] (2.5-4.8)
Cattle (n=12)	4.27 (3.3-5.0)	3.68 (2.8-4.4)
Pig (n=12)	3.88 (3.2-5.1)	3.58 (2.8-5.3)

[a, b] Significant difference ($P < 0.05$)

2.4 Concluding remarks

Our findings confirmed previously reported marked variability of microbial recoveries from carcasses when using wet-dry swabbing sampling method. In addition, the average recoveries obtained for swabbing were lower than, and not directly correlated with, the recoveries obtained using the excision method.

The factors influencing variability of swabbing recoveries in this study included two-way bacterial transfer between the swab and the meat surface (i.e. carcass-to swab-to carcass) and incomplete release of bacteria from the swab into diluent during homogenisation.

In the present study, reducing the effects of carcass-microflora-related variabilities through use of artificial inoculation did not result in a marked overall improvement in swabbing efficiency. This finding indirectly confirms the importance of inherent factors related to the swabs and/or the swabbing technique that were used for sampling. In spite of the inferiority of the swabbing method as compared with the excision in terms of bacterial recovery, the former is clearly preferred by the meat industry for carcass appearance reasons.

Therefore, a large-scale evaluation of the two carcass sampling methods has been further undertaken under commercial conditions (Chapter 3).

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3. Determination of field-performance of carcass sampling (wet-dry swabbing) method under commercial conditions and development of sampling plans/performance criteria for red meat abattoirs (OBJECTIVES 4 & 5)

3.1 Introduction

Traditional visual inspection of carcases by trained personnel is not an effective strategy for protecting consumers against meat-borne infections and physical and chemical hazards (3,4,20). A HACCP-based approach to production and processing is better suited for the manufacture of hazard-free meat (3). In keeping with decisions taken previously by regulatory authorities in the USA, Australia and New Zealand; EU Commission Directive EC/471/2001 became law for all red meat slaughterhouses in the EU in June 2003. An important change introduced by this new law was that all red meat slaughterhouses within the European Community were obliged to operate according to HACCP principles. Microbiological sampling and testing of carcasses was also introduced to verify that HACCP schemes were effectively monitoring plant processing conditions. These verification samples for carcasses are collected according to strictly defined sampling protocols and frequencies using an excision-based sampling method. In the UK, excision sampling is not popular and is practiced by less than 4% of red meat plants (Hutchison and Avery, unpublished) because the physical damage caused is unsightly and reduces the value of the sampled carcass (14). A number of alternative methods for carcass sampling have been described previously including a variety of disparate protocols collectively termed swabbing (6,7,9,14) and a number of surface contact methods (16). In the UK, one of the most widely practiced sampling methods for HACCP verification purposes is wet-dry swabbing (Hutchison and Avery, unpublished) using cotton-tipped stick swabs. A survey of over 100 full throughput red meat plants in the UK revealed that this method is used in >95% of UK red meat plants for HACCP verification purposes (Hutchison and Avery, unpublished).

The relationship between wet-dry swabbing and excision-based sampling is currently unclear. Although a number of workers have concluded that excision recovers significantly higher numbers of bacteria from meat surfaces than wet-dry swabbing (6,8,9), a number of explanations have been reported as the cause (4). Bacterial attachment to meat, growth and hence recovery, can be influenced by pH, the length of time that the microorganisms have been resident on the carcass and the temperatures that are used for chilling and drying (2,4,18). Furthermore, the abrasiveness of the swab material used for sampling (9) and the vigour and length of time used for stomaching and blending (19) all influence bacterial numbers measured from meat surfaces. Although EU Directive EC/471/2001 stipulates that excision sampling should be used for HACCP verification purposes in red meat plants, it makes provision for alternative sampling methods to be used provided they have been demonstrated as fit for purpose. The aims of this study were to assess the appropriateness of swab-based sampling; to examine potential reasons for the variability of microbial counts observed during carcass processing; and to determine the suitability of EU-specified indicator organisms as process control indicators for HACCP verification purposes in UK red meat plants.

3.2 Materials and Methods

Animal species examined

The four sites that were sampled varied with carcass species. For bovines, the sites were the rump, flank, brisket and neck. Sampling sites for ovines were the flank, brisket, breast and the lateral surface of the thorax. Porcine carcass sites were the ham, back, belly and jowl.

Excision sampling

Excision-based sampling involved removing a sliver of tissue of 5 cm^2 area and 2 mm thickness from the four sites on each carcass sampled. A flame-sterilised stainless steel borer (2.52 cm diameter) was firmly pressed into the

carcass surface and twisted to make an initial cut to a depth of ~3 mm. A sterile disposable scalpel (Swann Morton, Sheffield, England) and forceps (Scientific Lab Supplies, Nottingham England) were used to cut the tissue sliver free from the carcass. For pooled excision (PE) the four tissue slivers excised from each carcass were stored in a single 304 mm x 177 mm sealed polythene stomacher bag (Seward, Thetford, England) on crushed ice until the commencement of microbiological analyses.

Wet-dry swab sampling

Jumbo head cotton swabs (Sterilab Services, Harrogate, England) were used for wet-dry swab sampling. Samples were collected within an area of either 100 cm^2 (bovines) or 50 cm^2 (ovines and porcines) and these sampling areas were delineated by alcohol-sanitised stainless steel templates. The carcasses were swabbed at the same four sites listed above. For each sampling area, a swab was moistened in maximum recovery diluent [MRD; Bacteriological Peptone (Oxoid L37) 1 g, sodium chloride 8.5 g to 1000 ml], and then rubbed firmly across the carcass surface using ten strokes in each of the horizontal, vertical and diagonal directions. Swabs were rolled between the thumb and index finger as they were rubbed across the carcass surface. Immediately after rubbing with the MRD-moistened swab, the procedure was repeated within the same template using a dry swab. For individual swabbing experiments, each pair of wet and dry swabs was combined into a single sample in a sterile container containing 20 ml MRD and stored on crushed ice. For pooled swabbing (PS) experiments, the four wet-dry swab pairs from each carcass were combined into a single sterile container containing 80 ml MRD, stored on crushed ice and analysed as a single sample.

Microbiological examination of samples

Total aerobic viable counts (TVC) and *Enterobacteriaceae* counts were determined by standard plate count methods according to the criteria specified by ISO 4833:1991 (10) and ISO 5552:1997 (11), respectively. Briefly, microbiological analyses involved the addition of 100 ml of peptone

saline [10g protease peptone, 5g NaCl, 9g $Na_2HPO_4.12H_2O$, 1.5g KH_2PO_4, to 1000ml] to each stomacher bag containing excised slivers of tissue, followed by homogenisation for 1 minute using a stomacher (Model number BA 6021, Seward, UK). Individual wet-dry swab samples were weighed and their volume adjusted to 25 ml with MRD. Pooled swab sample volumes were adjusted to 100 ml with MRD. The containers containing the swabs (individual or pooled) were vigorously vortexed (Model SGP 202 O1OJ Fisons, Ipswich, UK) for 1 min. Each sample homogenate (excision or swab) was then diluted decimally in peptone saline and 1 ml aliquots added to appropriately labelled Petri dishes. For TVC, 15 ml of tempered (46°C) PCA [Oxoid CM325] was added to each Petri dish, mixed and allowed to harden. For *Enterobacteriaceae*, 15 ml of tempered (46°C) VRBGA [Oxoid CM485] was added, mixed and allowed to harden as before. TVC were incubated at 30°C for 72 h before colonies were counted. *Enterobacteriaceae* were incubated at 37°C for 24 h. Confirmation of *Enterobacteriaceae* was by oxidase testing and the ability to metabolise glucose. Bacterial numbers on decimally-diluted plates were converted into CFU cm^{-1} according to the criteria described by ISO 6887-1:1999 (12).

<u>Comparison of excision and swabbing sampling methods under commercial abattoir conditions</u>

For bovine carcasses, 49 visits were made to eight different cattle plants spread throughout England and Wales, and between 18 and 30 carcasses were sampled by either pooled swabbing (PS) or pooled excision (PE) during each visit. A total of 676 carcases were sampled by PS, and 676 carcases by PE. For ovine carcasses, 11 separate visits were made to seven different sheep plants spread throughout Scotland, England, and Wales and between 16 and 20 carcasses were each sampled by either PS or PE at each visit. The total number of ovine carcasses sampled were 94 (PS) and 94 (PE). For porcine carcasses, ten separate visits were made to eight different plants spread throughout England and Wales, and between 16 and 20 carcasses

were each sampled by PS or PE at each visit. The total number of porcine carcasses sampled were 88 (PS) and 88 (PE).

All carcass samples were collected under normal processing conditions in commercial slaughterhouses midway through daily production. For all species, samples were collected from carcasses after final inspection but before carcasses were chilled, normally from the side rail. The carcasses that were selected and the side that were sampled were chosen randomly. Sampling of near-consecutive carcasses was undertaken alternately by excision or swabbing from different carcases. Thus the first carcass selected was by excision, the next was by swabbing. Species-specific sampling sites and sample collection methods are described above.

Comparison of pooled and individual swab samples from different sites of the carcass at commercial abattoirs

For this part of the study, 84 bovine, 105 ovine and 80 porcine carcasses were sampled during 10, 11 and 10 commercial plant visits respectively. Although 105 ovine carcases were sampled, *Enterobacteriaceae* levels were determined in only 88 of these 105 samples. Wet-dry swab samples were collected from the four species-specific sites on carcasses and the pair of swab pairs from each site were stored separately as described above. At the laboratory, samples from each sampling site were processed individually. After vortexing, a 5 ml volume was removed from each of the four individual homogenates originating from a single carcass and these volumes combined and vortexed again to generate an artificially pooled sample. The homogenates from the individual wet-dry swab samples and the artificially pooled sample were examined for TVC and *Enterobacteriaceae* numbers using the methods described above. The microbial counts determined in the artificially pooled sample were compared with the arithmetic mean microbial counts calculated from the results of the four corresponding individual samples using linear regression.

Extended trend analyses for bovine species

Three full-throughput (>3000 carcases per week) bovine processing plants, were each visited once per week for a period of 13 continuous weeks. The plants were geographically proximal and the furthest distance between two plants was approximately 100 miles. During each visit, a minimum of 14 carcases were sampled by excision and a minimum of a further 14 by pooled swabbing. Samples were analysed for TVC and *Enterobacteriaceae* as described above and the mean log bacterial counts obtained from the first seven carcases sampled were compared to bacterial levels from the remaining seven. All samples were collected according to the criteria specified by EU commission decision EC/471/2001 and were acceptable as legitimate HACCP verification samples to British government slaughterhouse inspectors.

Assessment of microbiological variation of adjacent carcasses at commercial abattoirs

The individually-collected swab samples were further analysed to determine the variation between near-adjacent carcases on a single processing line. The results from each plant visit were logged, grouped by sampling site, and the geometric averages and standard deviations were calculated. The coefficients of variation (CV) were also calculated as percentage standard deviation divided by mean log bacterial count.

Statistical analysis

Wilcoxin Signed Rank tests (StatsDirect, Cheshire, UK) for non-parametric data and paired t-tests (StatsDirect) for normally distributed data were used to compare bacterial levels between excised and swab-collected samples and individual and pooled samples. For all tests, the confidence level for significance was 95% unless otherwise stated. Pearson Product-Moment correlation coefficients (Microsoft Excel 2000, Redmond, WA) were used to presumptively identify relationships between the bacterial counts obtained by

individual and pooled swab samples. Curve fitting using the least squares algorithm was used to assess the strengths of any presumptive relationships. Linear regression was also used to determine relationships between wet-dry swabbing and excision sampling.

The uncertainty associated with the results produced by each of the microbiological analyses methods used in the current study was determined using the critical differences method described originally by the Nordic Committee on Food Analysis (21). This methodology was devised originally for analytical chemical analyses and so adaptations to make it suitable for bacterial numbers were undertaken. Modifications to the protocol included log-transformation of the data used for the uncertainty calculations in keeping with the practices established by a number of previous studies (5,15,17). To facilitate log transformations, samples which did not test positive for bacterial numbers were recorded as 0.1 CFU cm^{-2} which is half the theoretical limit of detection of the analyses methods. Bovine carcasses were sampled by PE (n=12) and PS (n=12) and analysed in duplicate by two different technicians at the same time but in physically different laboratories using media and diluents from different manufacturers. A range of laboratory equipment (e.g. pipette guns, water baths, Petri dishes, incubators) were used. Samples were analysed for TVC and *Enterobacteriaceae* as described above. Differences between the two sets of analyses were used to calculate the relative repeatability standard deviation (21). Values that were further converted to an expanded measurement of uncertainty used a coverage factor of two which corresponded to a confidence level of 95% (21).

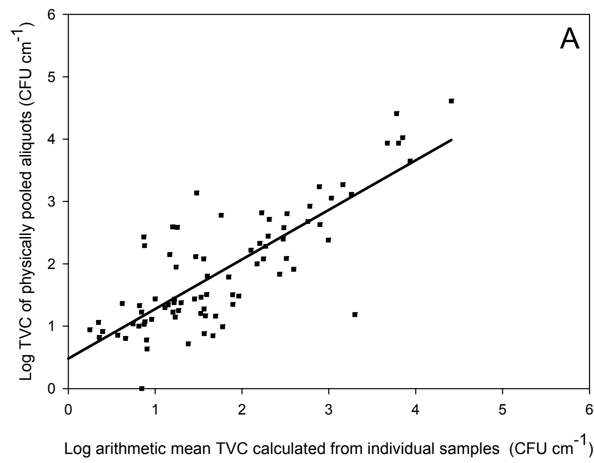
3.3 Results and Discussion

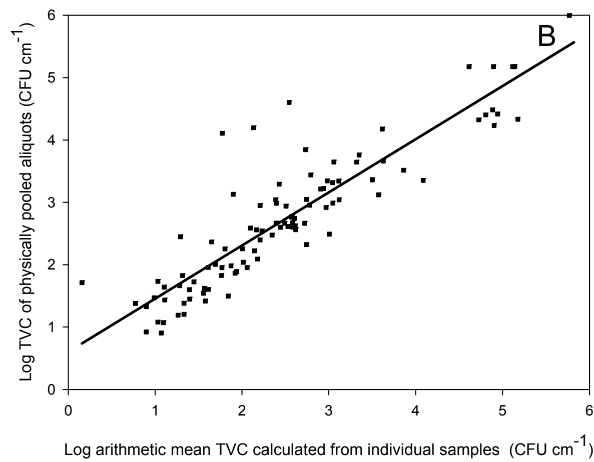
Comparison of pooled and individual swab samples from different sites of the carcass at commercial abattoirs

The relationship between wet-dry swab samples collected and analysed from individual sites on carcases and PS was investigated (Figure 1). Significant

correlations for all species ($P<0.001$) were detected by Pearson Product-Moment correlation coefficients (Table 1). Although the relationship between individual and PS for bovines and porcines was lower than that observed for ovines, analyses of the log-transformed counts using a paired t-test revealed that there were no statistically-significant differences between the mean of the individual samples (AM) and the count obtained from the artificially-generated pooled sample (PC) for any species. For ovines, there were a number of samples obtained from a single plant that contained elevated numbers of bacteria (Figure 1B). These samples skewed the distribution of the logged bacterial counts for ovine carcases and so further comparisons were undertaken using a Wilcoxin Signed Rank Test. As might be expected for samples which had a correlation coefficient (r) of 0.9, there were no statistically-significant differences between AM and PC for ovines.

The gradient of the lines formed by plotting AM against PC was around 0.8 for all three species (Table 1). This indicates that, although there is a linear relationship between AM and PC, that this relationship is not one-to-one because the gradient is not 1.0. The intersection of the fitted line across the PC axis was at around 0.55 for all species (Figure 1). This finding suggests that the pooled sample methodology was more effective at isolating bacteria when there were low numbers of bacteria. This is supported by the observation that when the regressions were constrained to pass through zero, and thereby lessening the impact of the low count data on the magnitudes of the slopes, the gradients were 1.00, 1.05 and 0.98 for cattle, sheep and pigs respectively. A possible explanation for this observation is that the extra pipetting and vortexing stages involved in generating the pooled samples could have resulted better in separation of aggregates of cells thereby giving rise to more plate colonies. The theoretical thresholds of detection for AM and PC are identical (0.125 cells cm^{-2} of carcass surface) and thus the result cannot be explained by differences in the detection abilities of two methods.





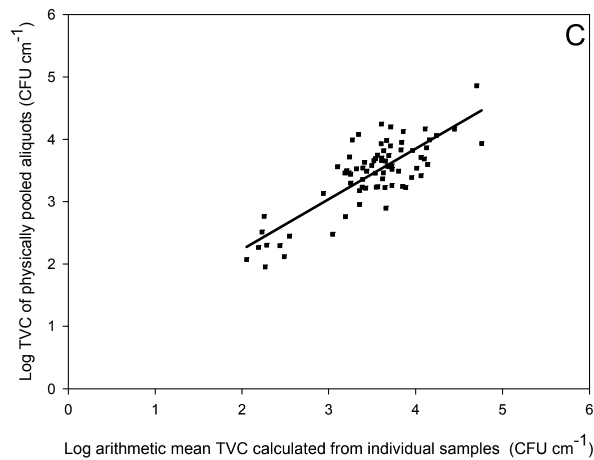


Figure 1: The relationship between the logarithm to the base 10 of the arithmetic mean TVC of individually collected and analysed wet-dry swab samples from four sites on bovine (A), ovine (B) and porcine (C) carcasses and the count obtained, also logarithmically transformed, from a PS generated by combining homogenate from the four carcass sites.

Carcass species	n	Number of plant visits made	r	Linear regression equation	(R^2)	Significantly different ($P<0.05$)
Cattle	84	10	0.809	PC = 0.785AM + 0.525	0.66	No
Sheep	105	11	0.903	PC = 0.854AM + 0.601	0.82	No
Pig	80	10	0.810	PC = 0.787AM + 0.615	0.65	No

Table 1: Determination of the relationship between individual and PS for TVC on carcasses under commercial operating conditions. Carcasses were sampled using a pair of wet-dry swabs for each of the EU-described carcass sampling sites. An artificial pooled sample was generated by combining an aliquot from each of these 4 individual samples. AM is the mean of the individual samples and PC is the pooled sample count. n is the number of carcasses sampled, r is the Pearson correlation coefficient that identifies presumptive relationships between datasets, R^2 is an indication the strength of the relationships. The right hand column refers to results of a paired t-test/Wilcoxon Signed Rank Test, see ' Results and Discussion.'

The majority (>95%) of full-throughput red meat processing plants in the UK undertake pooled swab sampling for HACCP verification purposes (Hutchison and Avery; unpublished). Results from these experiments are important because one method that plants can use to investigate higher bacterial counts from their processing lines is to switch from pooled sampling to individual sampling. Individual sampling can determine if elevated carcass bacterial counts result from higher levels at a single sampling site and thereby provide clues to the part of the process that requires corrective action. The results of these experiments have shown that the mean of four individual samples is not significantly different from a pooled sample. Therefore plants that switch to individual sampling to identify processing problems do not need to take additional pooled samples in order to meet legislative obligations. In addition, a number of British processors have historical process control data generated by sampling individual carcass sites. Although this data was generated before the legislatively-standardised protocols, our findings allow the retroactive

conversion of such data which is of benefit for long-term trends and the identification of seasonal variations.

<u>Comparison of excision and swab sampling methods using normally-processed carcasses at abattoirs operating under commercial conditions</u>

Figure 2 shows a comparison of bacterial counts from samples collected by excision or wet-dry swabbing. Although the porcine TVC comparisons depicted in Figure 2E show a loose trend that may be indicative of a relationship, the majority of the comparisons shown in Figure 2 clearly have little correlation. In particular, Figure 2A has a large number of high-value outlying points which are likely to confound comparisons. Furthermore there are a number of points that lie on or near either the x- or y- axis of the plots and again these low outliers make it unlikely that relationships between the two sampling methods exist. Although simple graphing of experimental data in formats similar to Figure 2 can be useful to determine obvious correlations between groups of data, we also used linear regression to quantitatively measure the strength of the relationships between excision and swabbing. The least squares-based straight lines that best fit the results are shown on Figure 2, and a summary of the regression equations for all three species is presented as Table 2.

The R^2 values shown in Table 2 are an indication of the quality of the fit between swabbing and excision. Since the R^2 values are low (<0.45) for both TVC and *Enterobacteriaceae* for each of the three species examined, we conclude that the bacterial numbers measured from samples collected by wet-dry swabbing are not related to those on near-consecutive carcases sampled by excision. Similar findings using smaller numbers of carcases derived from fewer processing plants have been found previously (13).

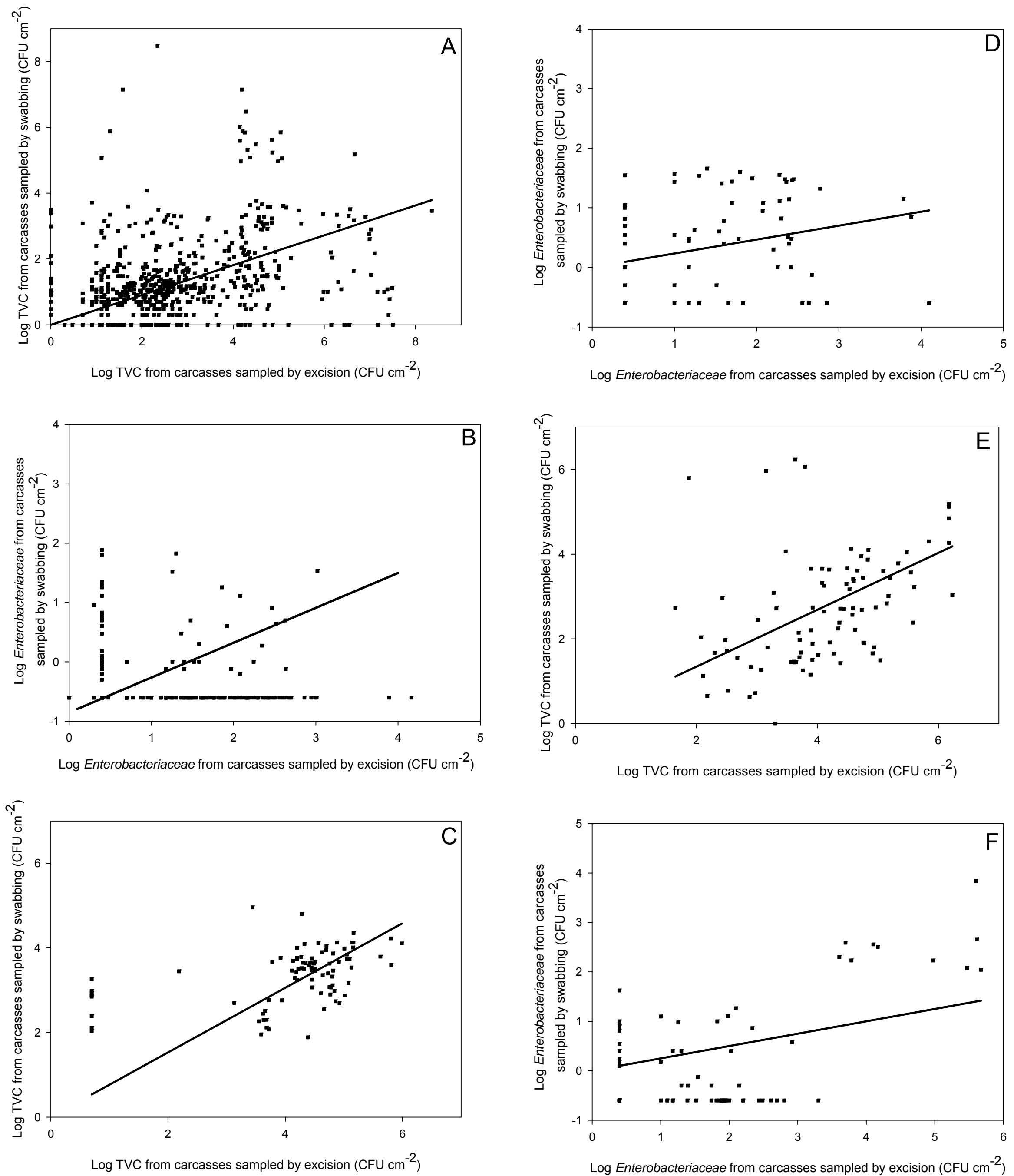


Figure 2: A comparison of total aerobic viable counts (A, C, E) and *Enterobacteriaceae* (B, D, F) obtained from near-consecutive bovine (A, B), ovine (C, D) and porcine (E, F) carcasses sampled by excision or wet-dry swabbing under commercial processing conditions. TVC are total aerobic viable counts.

Species	Total number of carcasses sampled	Number of plant visits made	TVC		*Enterobacteriaceae*	
			Regression equation	R^2	Regression equation	R^2
Bovine	1352	49	S=0.240E + 0.858	0.09	S = 0.0777E - 0.5688	0.02
Ovine	188	11	S= 0.627E + 0.201	0.27	S = 0.4847E - 0.6453	0.41
Porcine	176	10	S = 0.239E + 2.360	0.21	S = 0.3682E - 0.2706	0.19

Table 2: The relationship between bacterial counts obtained from carcasses processed under commercial conditions and sampled by either excision or swabbing. S and E are results of analyses taken by swabbing and excision respectively.

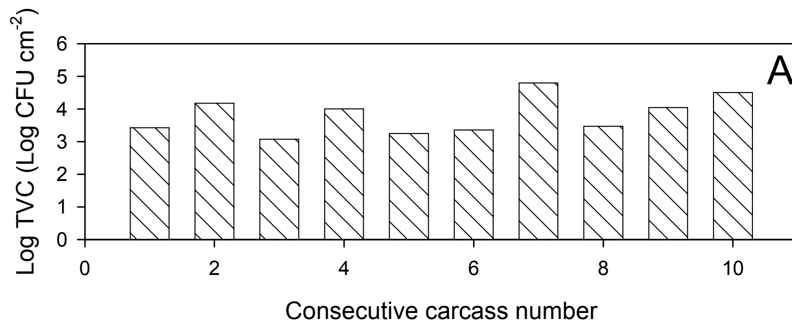
Measurement of the uncertainty associated with the bacterial analyses methods

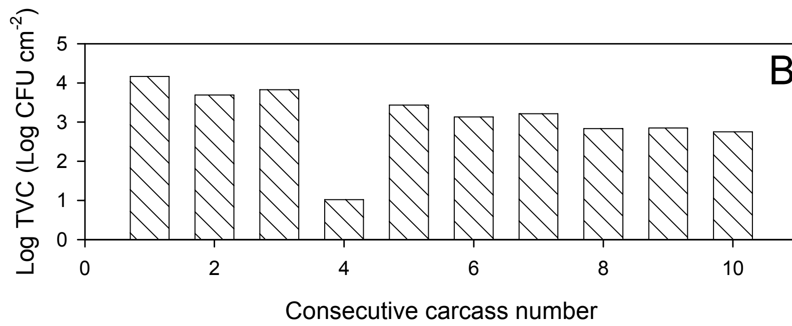
The measurement of uncertainty of the analyses methods have previously not been investigated as a reason for tenuous correlations between swabbing and excision. In comparative studies such as this, it is important to consider the accuracy of the bacterial measurements that are being made because large measurement errors when determining bacterial numbers can confound the identification of significant relationships. For this reason we undertook some measurements of the uncertainty associated with the laboratory analyses used in these studies using the critical differences method. Bacterial numbers were log-transformed for uncertainty calculations in keeping with established practices (17). The uncertainties measured for TVC and expressed as geometric relative standard deviations ($GRSD_r$) were 0.174 and 0.414 for excision and swabbing. Although there was an unusually large difference between the two $GRSD_r$ for the same analyses method, the

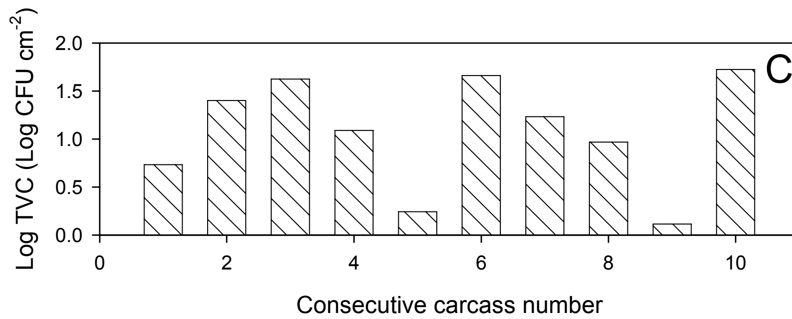
bacterial levels for TVC for swabbed samples were, on average, 50% of those obtained by excision. Previously it has been shown that lower analyte concentrations are more difficult to measure accurately and hence are subject to higher uncertainties (21). For similar reasons, the $GRSD_r$ calculated for rarely isolated *Enterobacteriaceae* were 0.669 and 0.462 for excision and swabbing respectively. Using the coverage factor of two recommended by Wood *et al.* (21), the uncertainty associated with excised *Enterobacteriaceae* is larger than the measured value (i.e. the potential measurement errors can be larger than the measurement itself).

Non-uniform distribution of bacteria between near consecutive carcases

Critical inspection of the bacterial counts observed from PS and individual wet-dry swab pairs from the four sites on carcases indicated that there could be a high degree of variation between the counts present on the surface of individual red meat carcasses closely positioned on commercial processing lines. A typical set of swab-sampled results drawn from a selection of plants is shown as Figure 3. On one occasion, there was a 1.5 Log CFU cm^{-2} difference between adjacent pooled swab-sampled porcine carcases (Figure 3A, carcases 6 and 7). A separate visit to a different porcine processor showed that the hams of two near-consecutive carcases had difference of over 2.5 Log CFU cm^{-2} in their TVCs (Figure 3B). Large differences in counts at the same site was not restricted to porcines. Similar differences were observed for both bovines and ovines (Figure 3C and 3D respectively). It is likely that the significant levels of variation in microbial numbers on the carcass surfaces was a contributory factor which helps explain why we were not able to demonstrate a true linear relationship between swabbing and excision. The reasons for this observation however are unclear. A plausible, though unlikely, explanation is that the processes in all of the plants that we visited were out of control. Another possibility is that the premise underlying carcass sampling for control purposes requires review. European Commission Directive EC/471/2001 requires that carcases be sampled because after removal of hide or fleece, or scalding of porcines, the carcass surface should be near-sterile. As the carcases move through the various







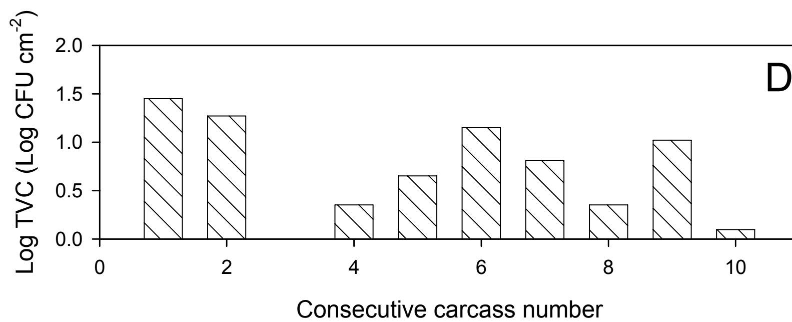


Figure 3: A representative selection of bacterial numbers obtained using swab sampling of carcases at different processing plants. Graph A shows a typical set of total aerobic viable counts (TVC) obtained from near-consecutive porcine carcasses sampled by pooled sampling from a single processing line. Graph B shows TVCs obtained from the hams of near-consecutive porcine carcasses from a different processing line. TVCs from the briskets of bovines and the flank of ovines are shown as C and D respectively.

stages of processing, they will acquire bacteria from the plant environment (i.e. equipment, personnel, atmosphere etc) and thus bacterial levels on the final carcass should give an indication of overall process hygiene. An unstated assumption for this tenet is that deposition of bacteria from the environment onto carcass surfaces is uniform. Currently it is not clear whether this is actually the case.

Prevalence of marker organisms

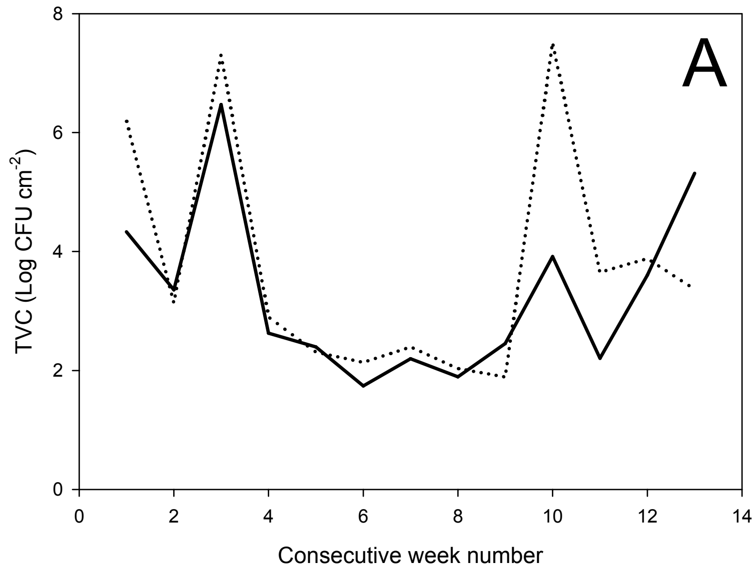
It is likely that the low prevalence of the *Enterobacteriaceae* on carcases was a contributory factor towards, but was not exclusively responsible for, our inability to correlate *Enterobacteriaceae* levels in samples collected by swabbing with those collected by excision because it is not possible to directly compare samples where no detections were made. A summary of positive detections of the indicator organisms on carcasses and their levels is shown as Table 3. Although EU commission decision EC/471/2001 contains performance criteria for *Enterobacteriaceae* levels on carcases, our experience during the current study was that this class of faecal indicator bacteria were not ubiquitously encountered on carcass surfaces in British processing plants. According to the criteria specified by Gill and Jones (9), at least 80% of samples should test positive for a bacterial indicator before it can be regarded as a potentially effective process monitor. Similar prevalences and conclusions regarding the appropriateness of *Enterobacteriaceae* as a process control indicator have been reported previously for cattle from a study involving 36 carcasses in an Irish plant (13). TVC were almost-ubiquitously recovered from all three species at high enough levels to be useful (Table 3). This bacterial group satisfy the criteria specified by Gill and Jones (9) for effective process control markers.

		TVC				Enterobacteriaceae			
		Positive isolations (%)		Log Mean level (log CFU cm^{-1})		Positive isolations (%)		Log Mean level (log CFU cm^{-1})	
Species	n_{ex}:n_{sw}	Excision	Swab	Excision	Swab	Excision	Swab	Excision	Swab
Bovine	676:676	99.3	95.1	5.89	5.69	23.4	7.7	1.77	-0.13
Ovine	76:76	100	100	5.35	4.79	43.7	43.6	4.26	1.99
Porcine	88:88	100	100	4.84	2.57	54.5	55.6	3.76	0.86

Table 3: Isolations of total aerobic viable counts (TVC) and *Enterobacteriaceae* on carcasses in a selection of UK plants and their numbers. Calculations to determine the mean levels of bacteria included carcasses where no bacteria were detected by substituting a value of 0 for not detected.

Extended trend analyses and the implications of these findings for commercial processors

A selection of the three-plant weekly trend samplings are shown as Figure 4. The trends shown on Figure 4 are typical of those obtained for both sampling methods at all three plants. The trends are erratic and it was not unusual for results to increase or decrease several logs between one week and the next. An important observation of this part of the study was that even although all of the samples were collected according to the criteria specified by EC/471/2001, the carcases that were selected for sampling could have a big influence on the bacterial counts that were measured. The two sets of week 10 counts for Figure 4A and the weeks 3 and 12 for Figure 4B all vary by a minimum of 4 Log CFU cm^{-2}. In the EU, 5 Log CFU cm^{-2} is the unacceptable threshold for TVCs on HACCP verification samples collected by excision and 4.3 Log CFU cm^{-2} for samples taken by wet-dry swabbing (1). Thus the variation observed was of sufficient magnitude to change the hygiene band even though all of the samples were collected according to EU-specified criteria. It is unlikely that slaughterhouse processing conditions changed so significantly over the interval that the samples were collected because, typically, all of the samples were collected in less than an hour.



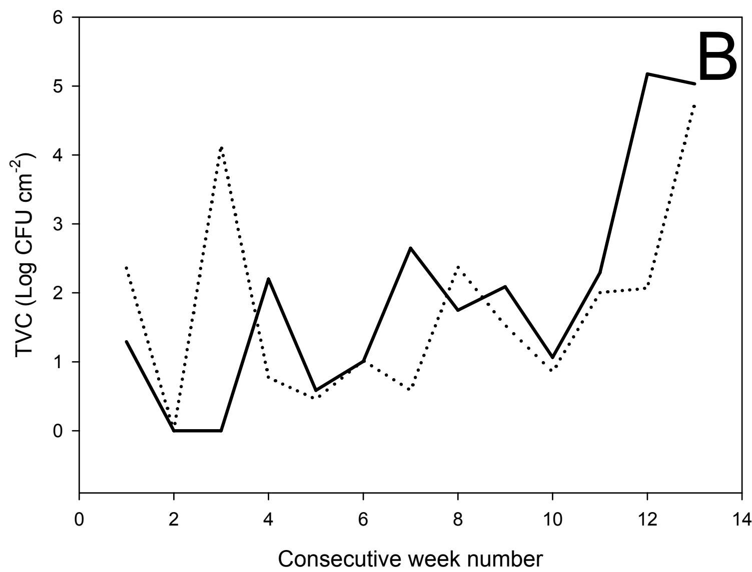


Figure 4: A selection of hygiene trends obtained from the weekly pooled sampling of bovine carcasses at three full-throughput slaughterhouses. Graphs depict the trends in total aerobic counts (TVC) at one plant where carcases were sampled by wet-dry swabbing (A) and a separate plant where trends were generated by excision sampling (B). The total number of samples collected by each sampling method were divided into two groups on the basis of order that they were sampled and plotted independently as the mean log of the first seven carcases sampled (solid line) and the last seven carcases sampled (broken line

3.4 Concluding remarks

The results observed during this study have shown that there are no significant linear relationships between carcass surface bacterial counts obtained by swabbing and excision. A contributory factor for this finding is related to our experimental design. Near-consecutive carcasses were sampled alternately by swabbing and excision because it was not appropriate to sample a single carcass by both methods. However, bacterial distribution on carcasses is not uniform and the carcasses that are selected for sampling make a significant difference to the bacterial counts that are measured. The reasons for such wide variations are complex and unlikely to have been caused by a single factor. An obvious, though unlikely, explanation is that all of the processes in the plants that were sampled during this study were not operating in a controlled manner. Also although the laboratory used for the microbiological analyses can influence bacterial counts, a single laboratory and three technicians were used to conduct these analyses.

EU commission Directive EC 471/2001 stipulates that weekly mean log trends should be used to ensure that hygiene of slaughter is controlled. Our results suggest that a simple mean log trend may not be an appropriate tool for HACCP verification purposes, because of the large variation between near consecutive carcases. There are a number of other process control systems that may be more appropriate including mean log trends calculated over several weeks and an ongoing cumulative sum measured against a baseline. However, in order to fully evaluate any system for process verification purposes, a larger dataset than the one used for this study is required because large numbers of samples will reduce the effect of the differences in the bacterial distribution and numbers between near consecutive carcasses on processing lines.

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4. Overall concluding remarks

The data collected in the survey, on current methods used by the meat industry to microbiologically verify HACCP in meat plants, were useful as an aid to develop a suitable practice method for HACCP verification for the purposes of Decision 2001/471/EC. It also assisted the overall project in having major importance in developing and recommending alternative EU-equivalent method(s) for microbiological testing of red meats of proven acceptability to the industry; sampling plans for high- and low-throughput red meat operators; and hygiene performance criteria in the context of HACCP/QA. The survey, however, confirmed that the information had not been available at that time, either in the scientific literature, or from current practice in meat plants, to allow decisions to be made on criteria for the microbiological monitoring in meat plants; so further experimental work was needed.

The project findings confirmed previously reported marked variability of microbial recoveries from carcasses when using wet-dry swabbing sampling method. In addition, the average recoveries obtained for swabbing were lower than, and not directly correlated with, the recoveries obtained using the excision method. The factors influencing variability of swabbing recoveries in this study included inherent variability of the distribution of microflora on, and its recovery by swabbing from, carcasses; two-way bacterial transfer between the swab and the meat surface (i.e. carcass-to swab-to carcass); and incomplete release of bacteria from the swab into diluent during homogenisation. In spite of the inferiority of the swabbing method as compared with the excision in terms of bacterial recovery, the former is clearly preferred by the meat industry for the carcass appearance reasons. Therefore, a large-scale evaluation of the two carcass sampling methods under commercial conditions was needed.

The results have shown that, under commercial conditions, there are no significant linear relationships between carcass surface bacterial counts obtained by swabbing and excision. A contributory factor for this finding is

related to our experimental design. Near-consecutive carcasses were sampled alternately by swabbing and excision because it was not appropriate to sample a single carcass by both methods. However, bacterial distribution on carcasses is not uniform and the carcasses that are selected for sampling make a significant difference to the bacterial counts that are measured. The reasons for such wide variations are complex and unlikely to have been caused by a single factor. An obvious, though unlikely, explanation is that all of the processes in the plants that were sampled during this study were not operating in a controlled manner. Also although the laboratory used for the microbiological analyses can influence bacterial counts, a single laboratory and three technicians were used to conduct these analyses.

EU commission Directive EC 471/2001 stipulates that weekly mean log trends should be used to ensure that hygiene of slaughter is controlled. Our results suggest that a simple mean log trend may not be an appropriate tool for HACCP verification purposes, because of the large variation between near consecutive carcases. There are a number of other process control systems that may be more appropriate including mean log trends calculated over several weeks and an ongoing cumulative sum measured against a baseline. However, in order to fully evaluate any system for process verification purposes, a larger dataset than the one used for this study is required because large numbers of samples will reduce the effect of the differences in the bacterial distribution and numbers between near consecutive carcasses on processing lines.

5. Scientific publications generated from the project

a) Refereed presentations on scientific conferences

Reid C.-A., Hutchison M., Small A., Comrie F., Wilson D. and Buncic S. (2002) Comparison of the excision and the swabbing techniques for microbiological sampling of carcasses at abattoirs. Proc. 48th International Congress of Meat Science and Technology, WHO, Rome, Italy, August 25-30, Proceedings, 954-955

Hutchison M., Wilson D., Reid C.-A., Johnston A.M., Howell M., Buncic S. (2003) Experimental and field evaluation of excision and swab-based sampling methods for porcine, ovine and bovine carcasses. 90th Annual Meeting of International Association for Food Protection (IAFP), New Orleans, August 10-13 2003, Proceedings, 109-110

b) Refereed papers in scientific journals

RICHARD PEPPERELL, CAROL-ANN REID, SILVIA NICOLAU SOLANO, MICHAEL L. HUTCHISON, LISA D. WALTERS, ALEXANDER M. JOHNSTON and SAVA BUNCIC (2004) Experimental comparison of excision and swabbing microbiological sampling methods for carcasses J. Food Prot., submitted

MICHAEL. L. HUTCHISON, LISA. D. WALTERS, SHERYL. M. AVERY, CAROL-ANN REID, DOUGLAS WILSON, MARY HOWELL, ALEXANDER M. JOHNSTON and SAVA BUNCIC (2004) A field comparison of wet-dry swabbing- and excision- sampling methods for microbiological testing of bovine, porcine and ovine carcasses at red meat slaughterhouses. J. Food Prot., submitted