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EXPERIMENTAL EVALUATION OF NON-MICROBIOLOGICAL METHODS FOR PROCESS HYGIENE ASSESSMENT IN ABATTOIRS

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Introduction

During slaughter and dressing of red meat animals, faecal contamination can be transferred to carcasses from a number of routes. These include the skin/hide of the animal, the gastrointestinal tract of the animal, and cross-contamination from environmental surfaces including processing equipment. In the UK there is a one in three chance that livestock-derived faecal material contains one of the five zoonotic agents (1) which cause over 80% of bacterial gastro-enteritis in humans.

To aid hygienic meat production in red meat slaughterhouses, the Meat (HACCP) Regulations, were implemented in the UK in 2002. These regulations require that carcasses and a proportion of the surfaces which come into contact with carcasses during dressing, must be sampled on a regular basis. Samples are examined for the total viable count (TVC) and numbers of *Enterobacteriaceae*, and the results give an indication of process hygiene. Analyses using conventional lab-based microbiological methods are currently exclusively used although these are costly. Another disadvantage of traditional microbiology is that the results take several days. If results were available in real time, and more rapid response to processing problems would be possible. The purpose of this study was to therefore evaluate rapid methods for their suitability in determining process hygiene in red meat slaughterhouses.

Several rapid methods are currently in use in the food industry. Commonly, such methods detect the presence of micro-organisms and/or food debris on food contact surfaces by detection of protein or ATP bioluminescence (2). Another rapid method being used extensively in the US in the meat industry is the detection of chlorophyll and its breakdown products on carcasses by measuring fluorescence when using the VerifEYE system (hand-held devices or a cabinet system). This detects emissions by chlorophyll a and its breakdown products at 675nm when excited by 420nm (3). Chlorophyll is broken down by the gut to fluorescent products which are present in the faeces of animals that have been fed a diet containing chlorophyll. Therefore any fluorescence detected on the carcasses indicates faecal, and hence possible bacterial, contamination.

In the UK rapid methods are not used extensively in red meat processing environments and the performance of non-traditional methods for the assessment of bacterial contamination is not known

Objectives

Two studies were carried out in a single low throughput abattoir in the South-West of England to evaluate the effectiveness of non-microbiological methods for the assessment of carcass and surface contamination during the dressing of cattle and pigs inoculated with chlorophyll, against the currently used microbiological methods

Methodology

Sample collection

Three cattle and three pigs were painted on the brisket and belly, respectively with a 50µg/ml solution containing chlorophyll a and b (50% of each), using a paint-brush method. Immediately after bleeding out, but before removal of the hide/entry into the scald tank, cattle and pigs were examined on the rump, flank, brisket and neck and the ham, back, belly and jowl, respectively using a hand-held version of the VerifEYE solo machine (Attec, UK) to detect the presence of chlorophyll. The same four sites on each animal species were sampled for *Enterobacteriaceae* and total viable count (TVC) by excising an area of hide from the cattle (10 x 10cm), and a piece of skin from the pigs (5cm^2) and placing into sterile stomacher bags. The animals were dressed using conventional techniques and the carcasses tested on the same sites as above using the VerifEYE solo machine and the excision method

Six environmental surfaces were collected after the completion of dressing of each species, these being: the roll-out ramp, beef pram, flayer, knife, apron wash and the splitting saw, for cattle, and: the dehairing machine (inside and outside), the polishing table, knife, apron wash and splitting saw, for pigs. Surfaces were sampled using: a wet/dry swabbing method (20cm^2 area) to determine *Enterobacteriaceae* and TVC, two protein detection methods - Flash sticks (Biocontrol, UK), and Pro-tect swabs (Biotrace Fred Baker, UK), Hygiena snapshot total ATP swabs (Hygiena International Ltd, UK), and the VerifEYE solo machine

After routine cleaning of the abattoir but before the start of slaughter the following day, the same six environmental surfaces for each species were examined, as outlined above

Laboratory analysis

From the excised pieces of hide a 5cm^2 area was aseptically excised and added to a sterile stomacher bag. To these and the excised pieces of carcass, 25ml of Maximum Recovery Diluent (MRD; Oxoid, UK) was added and the sample stomached for 2 min. The wet/dry swabs were added to 10ml MRD and vortexed for 1 min. All samples were further serially diluted in MRD and plated onto VRBG agar (Oxoid, UK) for enumeration of *Enterobacteriaceae*, and PCA (Oxoid, UK) for enumeration of TVC, using standard ISO methods

The Flash protein sticks and Pro-tect swabs were examined for colour change according to the manufacturers instructions. The Hygiena snapshot total ATP swabs were further processed according to the manufacturers instructions and the light output read in a luminometer

Analysis of results

The counts of *Enterobacteriaceae* and TVC were calculated for the hide, carcass, and environmental swab samples, to determine Log_{10} CFU/cm^2. For the two protein detection methods a score of between 0-4 was assigned to each sample depending on the degree of colour change, with 4 being the strongest. A score of 0-4 was also assigned to each sample examined using the VerifEYE solo machine, depending on the degree and extent of fluorescence found on the area (with 4 being the highest degree of fluorescence over the maximum area; subjective). The reading obtained on the luminometer from the total ATP swabs taken was displayed as Relative Light Units (RLU)

Results & Discussion

The data obtained for the carcasses of both cattle and pigs were calculated to show the difference in both microbial counts and chlorophyll levels between the hide/skin and the carcass (Figure 1). The average TVC count (Log_{10} CFU/cm^2, from four sampled areas of each species) from cattle hide was similar to that obtained from the pigs skin (Log_{10} 5.2 and 5.3, respectively). However, the same count detected on the finished carcasses of pigs was higher than that obtained on the cattle carcasses. When the difference between the carcass TVC count and the hide/skin TVC count was calculated for each species, the difference was higher with the cattle (Figure 1). This indicates that the transfer of bacteria from the hide to the carcass of cattle was lower than with pigs. The difference between the average levels of *Enterobacteriaceae* on the hide/skin and the carcasses of both pigs and cattle was similar, indicating similar levels of bacterial transfer from hide/skin to carcass. The average level of chlorophyll detected on the hide/skin and carcasses of cattle and pigs varied greatly (cattle: 2.9 and 0.9, pigs:1.1 and 0.2). The transfer of chlorophyll from the hide/skin to the carcass in both species also varied greatly (cattle: 2.0, pigs: 0.9), with the difference found to be greater in cattle (Figure 1). The difference in the level of chlorophyll detected on the hide/skin and carcasses from cattle showed a similar level as that obtained with the TVC count, and the corresponding difference in pigs, showed a similar level as obtained with the *Enterobacteriaceae* count.

The results obtained indicate that the transfer of contamination from the hide to the carcass was less with cattle dressing than with pig dressing, potentially indicating better process hygiene during the dressing of cattle in this abattoir. The results obtained also indicate that the detection of chlorophyll on carcasses may be a good indication of process hygiene, particularly in terms of the total bacterial load on cattle carcasses and bacteria from faecal origin on pig carcasses.

The environmental surfaces were examined pre- and post-cleaning to determine the effect of cleaning at reducing the levels of bacteria and visible contamination (Tables 1 & 2). With the cattle surfaces on average a 2 fold decrease in TVC count was observed which was also detected with the total ATP method and the Flash protein method (Table 1). Higher fold reductions were observe with the *Enterobacteriaceae* count (15 fold) and the Pro-tect protein method (8 fold). However, the chlorophyll levels detected using the VerifEYE solo machine showed no reduction between pre-and post-cleaning (Table 1). The reductions observed with the *Enterobacteriaceae* count from the surfaces sampled after pig slaughter were similar to those observed with the total ATP method (4 fold, Table 2). Both of the protein detection methods used showed a 2 fold reduction in contamination after the

surfaces had been cleaned (Table 2). The chlorophyll levels detected post-cleaning were higher than those detected pre-cleaning, and hence a reduction level was not obtained using this method (Table 2). The 15 fold reduction in TVC count observed post-cleaning was greater than the reduction detected with any of the other methods examined

The results obtained for the environmental surfaces indicate a potential correlation between measured total ATP, the Flash protein method, and TVC count, particularly with surfaces related to cattle slaughter. A similar correlation between the detection of microbial ATP and TVC from the surfaces of beef carcasses has already been found (4). Therefore, the detection of total ATP and protein may have a potential use to assess the effectiveness of surfaces cleaning in abattoirs

Conclusions

Overall, the results of the pilot study in an experimental abattoir indicate that the detection of chlorophyll on carcasses and methods to detect protein and total ATP on surfaces may have a potential for use in the assessment of abattoir process hygiene. However, these methods need to be further evaluated under commercial abattoir conditions

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Tables and Figures

Figure 1 – Difference between average detected *Enterobacteriaceae*, TVC and chlorophyll on hide/skin and carcass for cattle and pigs

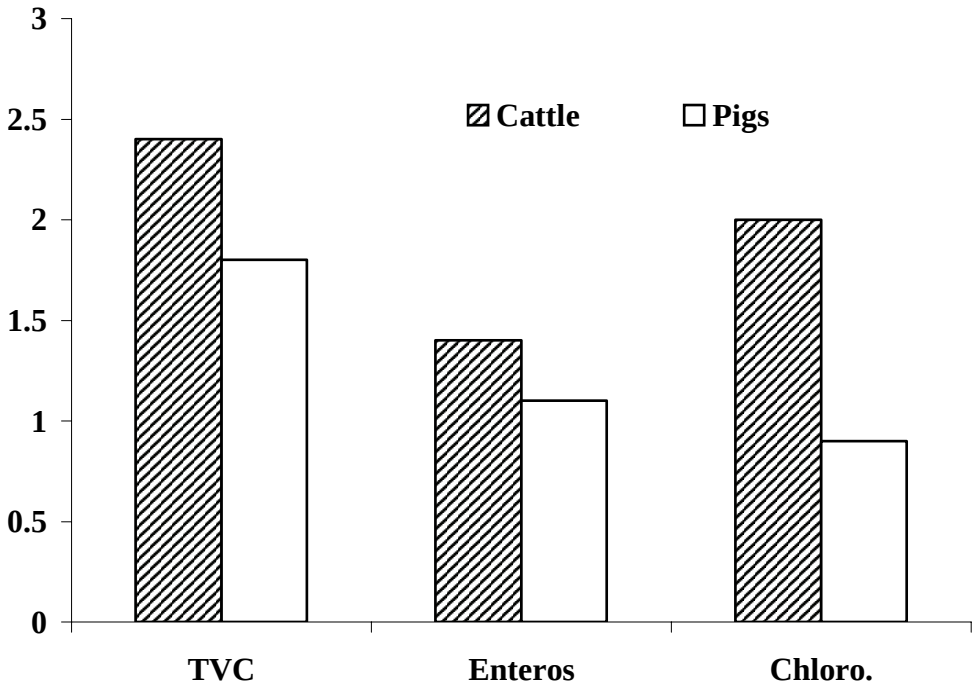

Table 1. Reduction in contamination parameters observed pre- and post-cleaning of surfaces in cattle slaughter

	TVC		Enteros.		VerifEYE		Total ATP		Pro-tect		Flash	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Roll-out ramp	4.8	3.1	2.7	0	3	3	928	1558	3	0	1	0
Beef pram	4.8	3.6	3.5	0	2	2	1860	2044	5	1	3	2
Knife	0	3.7	0	0	0	0	195	337	0	0	1	1
Flayer	3.8	1.5	0.2	0	1	1	16361	8305	3	0	1	1
Apron wash	1.5	1.9	0	0	1	0	1011	2	1	0	2	1
Saw	4.4	3.1	1.8	1.1	0	1	4566	504	0	0	3	1
Mean	3.2	2.8	1.4	0.2	1.2	1.2	4153	2125	1.7	0.2	1.8	1.0
Reduction	**2 fold**		**15 fold**		**0**		**2 fold**		**8 fold**		**2 fold**	

Table 2. Reduction in contamination observed pre- and post-cleaning of surfaces in pig slaughter

	TVC		Enteros.		VerifEYE		Total ATP		Pro-tect		Flash	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Dehairer (inside)	5.0	2.6	2.2	0	0	0	2760	903	3	3	3	1
Dehairer (outside)	1.1	1.2	0	0	0	1	120	542	0	0	1	1
Polishing table	4.6	0.2	2.2	0	0	0	1159	5696	1	0	4	3
Knife	2.9	4.2	0.2	0.7	0	0	48578	5724	3	3	3	0
Apron ash	0.8	1.0	0	0	0	0	2855	1547	0	0	0.5	2
Saw	1.9	0.5	0	0	0	1	8491	3791	2	0	2	1
Mean	2.7	1.6	0.8	0.1	0	0.3	10660	3034	1.5	1.0	2.2	1.3
Reduction	**14 fold**		**4 fold**		**Increase**		**4 fold**		**2 fold**		**2 fold**	