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Slaughter hygiene in European cattle and sheep abattoirs assessed by microbiological testing and Hygiene Performance Rating

Ole Alvseike[a,*], Elin Røssvoll[a], Ole-Johan Røtterud[a], Truls Nesbakken[b], Eystein Skjerve[b], Miguel Prieto[c,d], Marianne Sandberg[e], Gro Johannessen[f], Marianne Økland[f], Anne Margrete Urdahl[f], Sigrun J. Hauge[a]

[a] Animalia – Norwegian Meat and Poultry Research Center, P.O.Box 396, Økern, N-0513, Oslo, Norway
[b] Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P.O. Box 369, Sentrum, N-103, Oslo, Norway
[c] Department of Hygiene and Technology of Foods, Veterinary Faculty, University of León, 24071, León, Spain
[d] Institute of Food Science and Technology, University of León, 24071, León, Spain
[e] Department of Food and Veterinary Issues, The Danish Agriculture and Food Council, Axelborg, Axeltorv 3, 1609, Copenhagen, Denmark
[f] Norwegian Veterinary Institute, P.O.Box 750, Sentrum, N-0106, Oslo, Norway

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ABSTRACT

The aim of this study was to describe slaughter hygiene in a selection of ovine and bovine abattoirs in Europe, as assessed by microbiological testing and Hygiene Performance Rating (HPR) audits, and to compare the results obtained by these different approaches. Two types of microbiological testing were used: standardized study testing, which was similar in all abattoirs, and the abattoirs' own mandatory microbiological testing. Ten cattle slaughter lines were visited in Norway, Denmark, Germany, and Spain and 10 sheep slaughter lines were visited in Norway, UK, and Spain. HPR scores were obtained for each operation along the slaughter line and summed up into a total score (%). For the standardized study testing, gauze-cloth swabbing of approximately 800 cm^2 of cattle carcasses and 600 cm^2 of sheep carcasses was used on 25 warm carcasses at each slaughter line. In contrast, the mandatory microbiological testing conducted by the abattoirs used different types of equipment, methods, and analyses. Higher mean counts of *Enterobacteriaceae* and *E. coli* were obtained from sheep carcasses (-0.6 and -0.9 log CFU/cm^2, respectively) than from cattle carcasses (-1.4 and -2.5 log CFU/cm^2, respectively), and the numbers of *E. coli* detected on cattle carcasses were particularly low. A close relationship was found between the total HPR score and the *Enterobacteriaceae* and *E. coli* results obtained by the standardized study testing. For cattle, R^2 values were 0.69 and 0.62 for *Enterobacteriaceae* and *E. coli*, respectively, and for sheep the equivalent values were 0.62 and 0.60. The correlations between the HPR results and the microbiological results from the abattoirs' mandatory sampling were low, which could reflect fact that in 90% of the samples, bacteria tested were below the limit of detection with the methods used. This study reports the methodology and results from the first European baseline study on slaughter hygiene. The correlation between the HPR results and the standardized study testing of the carcasses suggests that HPR could be a useful proxy measure for improving slaughter hygiene and risk management.

1. Introduction

Since regulatory microbiological testing of carcasses cannot be used to guarantee 100% food safety in meat, the current strategy is to ensure that abattoir premises and procedures are sufficiently hygienic for the risk to be at an acceptably low level. Introduction of the Good Hygiene Practice (GHP) and Hazard Analysis Critical Control Points (HACCP) approach conceptualized auditing and microbiological testing as a way to verify that a Food Business Operator (FBO) had a hygienic food production system (Hulebak & Schlosser, 2002). Companies supplying export markets are required to adapt to the regulations of these markets, and the mandatory implementation of HACCP-based systems has been legislated by governments worldwide (Anon., 2015b; FSANZ (Food Standards Australia New Zealand), 2016; Govender, 2013; Tshabalala, 2011).

Microbiological testing of carcass hygiene has been systematized in legislation in the US and Europe since the 1990s (Brown et al., 2000). The testing regime is not intended to act as a control that guarantees the

* Corresponding author.
E-mail address: ole.alvseike@animalia.no (O. Alvseike).

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wholesomeness of individual carcasses or products thereof (Milios, Drosinos, & Zoiopoulos, 2014; Tortorello, 2003). For instance, the EU regulation 2073/2005 sets process hygiene criteria, in which acceptable limits for microorganisms (McKenna et al., 2002) and analytical reference methods are described (The Commission of the European Communities, 2005). In the EU, the reference method for carcass sampling is described in ISO 17604 (Anon., 2015a) as destructive sampling of hot carcasses to be analysed for *Enterobacteriaceae*. However, other methods can be used provided that equivalent results are obtained. In addition, indicator organisms other than *Enterobacteriaceae*, such as *Escherichia coli*, may be targeted (Capita, Prieto, & Alonso-Calleja, 2004; The Commission of the European Communities, 2005). European abattoirs use a wide range of different methods for carcass-hygiene surveillance (Røssvoll et al., 2017), and several efforts towards validation of different microbiological carcass sampling approaches have been made (Christensen, Baggesen, Soerensen, & Svensmark, 1999; Gallina et al., 2015; Gill & Badoni, 2010; Karr et al., 1996; Lindblad & Berking, 2013; McEvoy, Sheridan, Blair, & McDowell, 2004; Pepperell et al., 2005; Røssvoll et al., 2017). Nevertheless, adjusted limits for the microbiological criteria for the various methods have not been officially approved to date, and documented conversion factors between different sampling techniques are limited (Christensen & Olsen, 1999; Gallina et al., 2015; McEvoy et al., 2004; Røssvoll et al., 2017).

Testing regimes are costly for the meat industry and should have a positive contribution on public health and improved product quality. There is an opinion within the Norwegian meat industry, that slaughter hygiene has improved gradually and significantly during recent decades, with negative test results being by far the most common. However, the reliability of the microbiological results has been questioned (Vanderlinde, Jenson, & Sumner, 2005), and, likewise, whether the sampling and analyses used are sufficiently sensitive to detect faecal contamination of carcasses (Røssvoll et al., 2017).

Objective criteria and common scales imply that the results between different abattoirs should be comparable and useable for benchmarking and supporting fair competition. Baseline studies can be a helpful tool and contribute towards meeting the challenges, as well as assisting in identifying opportunities for improving food safety. National baseline studies of carcass hygiene of different species have been published (Anon., 1996, 2009, 2011b; Bohaychuk, Checkley, Gensler, & Barrios, 2009; Bohaychuk, Gensler, & Barrios, 2011; Ghafir, Dierick, & Zutter, 2008; Lindblad, 2007). EU has conducted International baseline studies for specific pathogens, e.g. MRSA in swine and *Campylobacter* in poultry (EFSA, 2009; Havelaar, Ivarsson, Lofdahl, & Nauta, 2013). In the USA, the Food and Safety Inspection Services (FSIS) has undertaken interstate baseline studies on different slaughter animal species (Anon., 1994, 1996, 2009, 2011b, 2011a, 2012). In Australia, the first national baseline study on beef carcasses was conducted in 1993 and has been regularly performed since then (Phillips, Bridger, Jenson, & Sumner, 2012; Phillips, Jordan, Morris, Jenson, & Sumner, 2006; Vanderlinde, Shay, & Murray, 1998). However, comparison of hygiene levels from different national studies has been hampered by methodological differences (Phillips Jordan, Morris, Jenson, & Sumner, 2006). To our knowledge, few, if any, international baseline studies on carcass microbiology have been undertaken in Europe, despite these being the tests most frequently applied, and thus being of greatest cost to the industry.

There are also other systems for assessing slaughter hygiene. In Australia, Meat Hygiene Assessment (MHA) systems are implemented in the HACCP-based Quality Assurance programmes (Singleton, 2002, p. 131), and trained company staff perform standardized process and product monitoring at specific steps in the processing line (Butler, Murray, & Tidswell, 2003). In the UK and South Africa, Hygiene Assessment System (HAS) was used to audit compliance at abattoirs (Govender, 2013). In Norway, a unique tool for assessing slaughter hygiene, Animalia's Hygiene Performance Rating (HPR) auditing system, has been used in abattoirs for the last 10 years (Anon., 2016b). We are not aware of any examples of scientific assessment of the hygienic impact of legislation and audits, although these would be very relevant (Enoe, Christensen, Andersen, & Willeberg, 2003). Here we describe what we believe to be the first attempt to correlate the results obtained from a purely slaughter-line auditing system with data on the microbiological contamination of carcasses, as ascertained by sampling and laboratory analysis.

Thus, the aim of this study was to describe slaughter hygiene in a selection of ovine and bovine abattoirs in Europe, as assessed by microbiological testing and by HPR audits. Two types of microbiological testing were used: standardized study testing, which was similar in all abattoirs, and the mandatory microbiological testing regime of the individual abattoirs.

2. Materials and methods

2.1. Slaughter lines

The study was conducted at 20 commercial slaughter lines, 10 for cattle in Norway, Denmark, Germany, and Spain, and at 10 for sheep in Norway, UK, and Spain. Within our limitations (economy and accessibility) abattoirs included were chosen to represent various slaughter lines in terms of capacity, line speed, technology applied, etc. In total, 14 abattoirs were visited in 2016–2017, of which six abattoirs had both cattle and sheep slaughter lines included in the study. The abattoirs slaughtered between 7000 and 720 000 cattle and between 20 000 and 1 000 000 sheep annually. Slaughtering speed varied from slow group slaughtering and up to 100 cattle carcasses per hour, and from 170 to 500 sheep carcasses per hour. The number of operators varied from 13 to 64 per slaughter line. Some of the main characteristics of the slaughter lines and slaughter techniques are shown in Table 1A (cattle) and 1B (sheep).

Table 1A
Some characteristics for the 10 cattle abattoirs in the study.

Cattle abattoir	Speed, carcasses/h	Operators, n	Sealing of rectum (bagging)	Rodding of oesophagus	Steam-vacuum	Carcass washing
1	25	13	Yes	Yes	No	No
2	35	22	Yes	Yes	No	No
3	15	16	Yes	Yes	No	No
4	20	13	Yes	Yes	No	No
5	20	14	Yes	Yes	No	No
6	15	22	Yes	Yes	No	No
7	20	14	Yes	Yes	Yes	No
8	60	31	Yes	Yes	Yes	Yes
9	40	17	No*	Yes	No	No
10	100	22	Yes	Yes	No	Yes

* Normally bagging was performed in this abattoir but not on the day of the audit.

Table 1B
Some characteristics for the 10 sheep abattoirs of the study.

Sheep abattoir	Speed, carcasses/h	Operators, n	Shearing	Sealing of rectum (bagging)	Rodding of oesophagus	Evisceration by two main methods, 1) or 2)	Steam-vacuum	Carcass washing	Neck cutting
11	500	64	No	No	Yes	1, sphincter muscle cut	No	Yes	No
12	200	22	Whole body, in lairage	No	Yes	2	No	No	No
13	260	27	Whole body, on-line	No	No	2	Yes	No	Yes
14	270	26	Whole body, on-line	Yes	Yes	1	Yes	No	Yes
15	210	19	Whole body, in lairage	Yes	Yes	1	No	No	No
16	240	26	Whole body, on-line	No	No	2	Yes	No	Yes
17	245	26	Whole body, in lairage	No	Yes	2	No	No	No
18	170	22	Whole body, in lairage	Yes	Yes	1	No	No	No
19	400	32	No	No	Yes	1, sphincter muscle cut	Yes	Yes	No
20	100	34	No	No	No	1, sphincter muscle cut	No	Yes	No

1) Evisceration hanging by the hind legs, head down, usually sealing the rectum with plastic bag after circum-anal incision, pulling the intestines out through the belly.
2) Evisceration hanging by the forelegs, head up, rectum cut when taking the intestines out, rectum removed the opposite way of the carcass as the intestines.

2.2. Carcass sampling

2.2.1. Standardized study sampling of carcasses

From each abattoir, 25 samples were randomly collected at the end of the slaughter lines prior to chilling, giving a total 500 carcass samples analysed. Sampling was conducted during the course of a single day for each abattoir. The swabbing area was chosen based on recommendations described in ISO 17609 (2015). For cattle carcasses, 800 cm^2 was sampled. The sampling sites were the thigh caudally and medially (200 cm^2), inside the pelvic cavity and outside caudally to the sirloin and around the tail set (200 cm^2), externally and internally to the xiphoid and sternum (200 cm^2), and laterally and medially to the foreleg (200 cm^2). The cattle carcasses were swabbed alternately on the right/left side for every second carcass. For sheep carcasses, an area of 600 cm^2 was sampled (Fig. 1). Sampling sites for sheep carcasses were lateral to the knees (100 cm^2), internal pelvic cavity (100 cm^2), external abdominal wall (100 cm^2), external anterior sternum (100 cm^2), lateral foreleg (100 cm^2), and laterally to the neck (100 cm^2).

Gauze-cloth swabbing was conducted by three different operators in different abattoirs, using a sterile medical gauze cloth (10 × 10 cm) (Mesosoft, Mölnlycke Health Care AB, Sweden) to which 10 mL sterile peptone salt diluent was added (pH 7.0 ± 0.2, Oxoid Ltd., Basingstoke, Hampshire, UK). The sampling area was swabbed using 10 horizontal and 10 vertical movements (approximately 20 s). When swabbing cattle carcasses, two gauze cloths swabs were used: one for the hind legs and pelvis, and one gauze cloth swab for the forepart and front legs, and both swabs were pooled into a single sample. Sheep carcasses were swabbed using one gauze cloth, and after swabbing the hind legs and pelvic areas, the cloth swab was turned and used on the other side to swab the forepart and front legs. After sampling, the swabs were placed in individual stomacher bags and kept refrigerated during transport to the analysing laboratory. Analyses were conducted at the Norwegian Veterinary Institute, Oslo, except for samples from Spanish abattoirs that were analysed at the Institute of Food Science and Technology, University of León, Spain, due to logistic considerations. All samples were analysed the day following sampling.

2.2.2. Abattoirs' mandatory routine testing

In addition to the standardized study sampling, the abattoirs' routine testing was performed. Sampling was conducted by the same personnel that usually do this sampling, and in the same way; same swabbing area, same technique, same equipment, same analysing laboratory etc. The only difference was that they were asked to sample 25 carcasses instead of the usual five carcasses. For cattle carcasses, the routine sampling was performed on the opposite halves of the same carcasses that had been sampled for the standardized study sampling, i.e. alternately on the left and right side of the carcasses. For sheep carcasses, the mandatory routine testing was conducted on other carcasses than those sampled for the standardized study sampling, because the swabbing areas on sheep carcasses were on both sides of the carcasses. The routine samples were collected either on warm carcasses or on cold carcasses after 24 h of chilling. One abattoir sampled according to the EU's destructive reference method (The Commission of the European Communities, 2005), and 15 abattoirs used alternative methods. These included swabbing with gauze cloth, sponge, sponge-stick or wet-dry double-swab technique. Table 2 shows the sampling regimes used in each abattoir.

2.3. Microbial analyses of standardized study samples

The standardized study samples, from both cattle and sheep, were homogenized for 30 s in 20 mL sterile peptone salt diluent using a stomacher (Laboratory blender, Stomacher 400, Seward, UK), and serially diluted. *Enterobacteriaceae* and *E. coli* were enumerated using SimPlate® *Enterobacteriaceae* and SimPlate® Coliforms/*E. coli* (BioControl Systems Inc, Bellevue, WA, USA). One mL from the

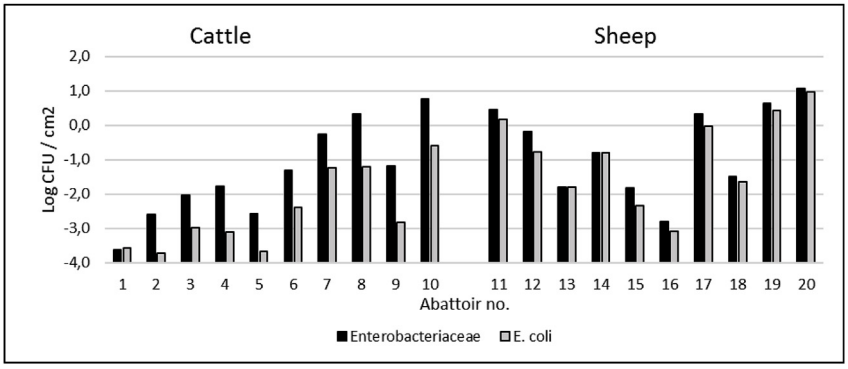

Fig. 1. *Enterobacteriaceae* (black bars) and *E. coli* (grey bars) mean log CFU/cm^2 per slaughter line (numbered from 1 to 20) from extended study tests. Results for cattle to the left and sheep to the right.

Table 2
Detection results from the abattoirs' routine testing from either Enterobacteriaceae results or *E. coli* and the results from the standardized study microbiological testing.

	Method	Sample area (cm^2)	Warm or cold carcasses sampled	Indicator bacteria analysed	Abattoirs' test (detected/total)	Study test *Enterobact-eriaceae* (detected/total)	Study test *E. coli* (detected/total)
Cattle abattoir							
1	Mesosoft cloth swab	3*100	Warm	*E. coli*	2/20	5/25	6/25
2	Sponge sticks	3*100	Cold	*E. coli*	1/25	18/25	4/25
3	Mesosoft cloth swab	4*100	Cold	*E. coli*	0/25	23/25	12/25
4	Mesosoft cloth swab	4*100	Cold	*E. coli*	0/25	21/25	12/25
5	Mesosoft cloth swab	3*100	Cold	*E. coli*	0/25	18/25	4/25
6	Mesosoft cloth swab	4*100	Warm	*E. coli*	0/5	25/25	20/25
7	Mesosoft cloth swab	3*100	Cold	*E. coli*	0/5	25/25	24/25
8	Destructive	4*5	Warm	*Enterobact-eriaceae*	1/5	25/25	23/25
Sheep abattoir							
11	Sponge (Polywipe)	1000	Warm	*Enterobact-eriaceae*	5/25	25/25	25/25
12	Mesosoft cloth swab	4*100	Cold	*E. coli*	0/25	25/25	25/25
13	Mesosoft cloth swab	3*100	Cold	*E. coli*	0/10	21/25	21/25
14	Sponge sticks	3*100	Cold	*E. coli*	14/25	25/25	25/25
15	Mesosoft cloth swab	3*100	Cold	*E. coli*	5/25	20/25	16/25
16	Mesosoft cloth swab	3*100	Cold	*E. coli*	0/25	14/25	9/25
17	Mesosoft cloth swab	4*100	Cold	*E. coli*	4/25	25/25	25/25
18	Mesosoft cloth swab	4*100	Warm	*E. coli*	3/25	24/25	22/25

appropriate dilution was placed in the centre of the SimPlate plating device, and 9 mL of a mixed nutrient agar with blue colouring was added at the same spot and the device rotated according to the manufacturer's instructions. The plates were incubated at 37 ± 1 °C for 24–28 h and read according to the manufacturer's instructions. For the *E. coli* analysis, wells with a change in colour from that of the background and that fluoresced when exposed to 366 nm UV light were counted as positive and converted into most probable number (MPN) of *E. coli* per swab/sample according to a counting range conversion table. Correspondingly, for the *Enterobacteriaceae* analyses, wells with a colour change from the background were counted as positive, according to the manufacturer's instructions. If all 84 wells demonstrated a positive reaction, the result was reported as > 738 MPN per plate.

2.4. Hygiene Performance Rating

All 20 slaughter lines were assessed according to the HPR-protocol developed by Animalia, Norway (Hauge, 2017; Røtterud, Gravning, G.E., Hauge, & Alvseike, 2019). The HPR is based on a visual, systematic evaluation of hygienic practices, performed by one trained external assessor. In accordance with the HPR protocol, factors that can affect slaughter hygiene in the operations along the slaughter line were assessed in detail, with particular focus on the operators' hygienic behaviour and risk handling of the carcasses, and each operation was given a score that was recorded electronically.

The HPR also involved detailed recording of activities from killing to grading of the finished carcasses. According to the HPR protocol, the assessment started in the morning, before the slaughtering started, with evaluation of the quality of washing and cleaning of the slaughter line, and observation of the preparation routines of the operators. Information on the quality (appropriateness, thoroughness and use of soap) and frequency of hand washing was collected, along with information on use of two-knives method with knife sterilizers (Taormina & Dorsa, 2007), and compliance with GHP especially at de-skinning and evisceration where hide/skin or leakage from intestines may contaminate the meat. For all slaughter operations, a major focus was unnecessary contact between the clean carcass and (dirty) hands or knives, equipment, furnishings, etc. Deviations and errors were counted and recorded, e.g., the percentage of rectums and intestines accidently punctured, the percentage of carcasses with remnants of hide/fleece after deskinning, unacceptable amounts of blood on carcass surfaces, and visible contamination. The "worst" observed practise of observed operational repeats was recorded, rather than the mean value of all the observed repeats. The condition of the building/premises, how the slaughter line was organized and managed, the use and sanitation of technical equipment, and working routines were other factors that were assessed. The management practices of the FBO and those of the Competent Authority's personnel were also included in the assessment, as these can have important effects on employees' motivation and the resulting work culture within the FBO. All observations were scored from 1 to 3, where 1 means "acceptable", 2 = "improvements necessary", and 3 = "not acceptable". Scores for each position were weighted

for hygienic impact and assumed risk (1, 3, 6 or 12) and economic consequences (1 or 2) that was determined on the basis whether the abattoir must invest a large sum of money (1) or whether a cheap quick-fix could be implemented (2). These scores were calculated into a final percentage value per operation, where 100% is considered to represent full compliance with the practise defined as "acceptable". The percentages for each operation were then calculated into a total HPR index for the slaughter line. Scores were also given for routines, facilitation, and management. For each abattoir a report was made available on-line containing results from the HPR assessment and recommendations where appropriate. These HPR indices were compared with the parallel microbiological results from standardized study swab samples obtained on the same day as the HPR assessment and also with the microbiological results from the FBO's own routine sampling.

2.5. Statistical analyses

Microbial data from the SimPlate analyses were transformed from MPN per sample to $\log_{10}$ per cm^2 for statistical analyses, and the microbial data from the abattoirs' routine testing were also log-transformed. Results below the detection limit were set at 0.1 CFU per sample and results " > 738" MPN per sample were set to "7380" for calculation of means. Data from HPR auditing and microbial testing were compiled in a spreadsheet in Excel® (Microsoft, Seattle, WA), and statistical analyses undertaken using Stata IC version 13 for Windows, (StataCorp, College Station, Texas). Analyses were performed using graphical techniques, means with 95% confidence intervals (CI) for each response variable and groups (cattle/sheep and abattoirs) were calculated, and differences between groups were tested by ANOVA and paired t-tests. Finally, simple linear regression analyses were performed using both *E. coli* and *Enterobacteriaceae* as response variables and HPR as explanatory variable to assess the relationship between HPR as a predictor for bacterial counts, and the explanatory power of the regression (R^2).

3. Results

3.1. Microbiological results from the standardized study sampling

Enterobacteriaceae were detected in 84% (210/250) of the cattle carcasses and 92% (229/250) of the sheep carcasses. *E. coli* was detected in 58% (145/250) of the cattle carcasses and 87% (218/250) of the sheep carcasses. For the 250 cattle carcasses in total, the *Enterobacteriaceae* mean value was −1.4 log CFU/cm^2 (SD 1.4, CI_{95} -2.4- −0.4) and the *E. coli* mean value was −2.5 log CFU/cm^2 (SD 1.1, CI_{95} -3.3- −1.7). For the 250 sheep carcasses, the results were higher ($p < 0.001$) with *Enterobacteriaceae* mean of −0.6 log CFU/cm^2 (SD 1.3, CI_{95} -1.6–0.3) and *E. coli* mean of −0.9 log CFU/cm^2 (SD 1.3, CI_{95} -1.8–0.0). The *E. coli* values for cattle carcasses were considered to be especially low. The *Enterobacteriaceae* and *E. coli* results for each abattoir are shown in Fig. 1. The *Enterobacteriaceae* means per abattoir varied from −3.6 to 0.8 log CFU/cm^2 and *E. coli* means varied from −3.7 to 0.6 log CFU/cm^2. In 19% (96/500) of the samples, the *E. coli* value was higher than the *Enterobacteriaceae* value. There was a larger difference in means between *Enterobacteriaceae* and *E. coli* for cattle (1.1 log CFU/cm^2) than sheep carcasses (0.3 log CFU/cm^2, $p < 0.05$).

3.2. Microbiological results from the abattoirs' routine testing

In this study, five of the abattoirs sampled warm carcasses and 11 sampled cold carcasses.

Results from 135 cattle carcasses and 185 sheep carcasses were obtained from the abattoirs' routine testing. Eight cattle and eight sheep slaughter lines shared their results, whereas four abattoirs did not provide their results to the study. The number of carcasses included in the abattoir sampling varied. A full parallel sampling of 25 carcasses was obtained from 11 slaughter lines, three abattoirs sampled five carcasses, one abattoir sampled ten, and one sampled 20 carcasses (Table 2). Testing was performed either by the abattoirs' personnel or by personnel from external labs.

Two abattoirs analysed for *Enterobacteriaceae*, which was detected in 20% of both the cattle samples (1/5) and the sheep samples (5/25). Fourteen abattoirs analysed for *E. coli* and 15 abattoirs analysed, in addition, for Total Plate Count. *E. coli* was detected in two out of seven cattle abattoirs, and in 2% of the samples. This occurrence was considerably lower than in the standardized study sampling ($p < 0.05$). For the same seven slaughter lines, *E. coli* was detected in 47% (82/175) of the cattle carcasses in the standardized study sampling. For sheep carcasses, *E. coli* was detected in four out of seven sheep abattoirs, which was a detection of 16% (26/160) of the samples. Compared with the *E. coli* results of the standardized study, this detection percentage was low. For the same seven slaughter lines, *E. coli* was detected in 81% (141/175) of the sheep carcasses in the standardized study sampling. The percentages of samples where *Enterobacteriaceae* or *E. coli* were detected varied from 0 to 56% between abattoirs. Mean value of Total Plate Count for cattle was 2.0 log CFU/cm^2 and 1.5 log CFU/cm^2 for sheep.

3.3. HPR results and their relationship with microbiological carcass hygiene analyses

The HPR results ranged from 43% in the abattoir with the poorest slaughter hygiene to 76% in the abattoir with the best slaughter hygiene. The mean value was 64% for all 20 slaughter lines; the mean for cattle was 67% (CI_{95}: 59–75), and the mean for sheep was 60% (CI_{95}: 52–68). Thus, HPR scores were higher (better hygiene), in abattoirs processing cattle than sheep ($p = 0.06$). The HPR scores for the sheep pre-skinning operations (47%) and evisceration (55%) were especially poorer ($p < 0.05$) than the equivalent results for cattle, which were 66% and 59%, respectively (Fig. 2).

The relationships between HPR audits and microbiological carcass hygiene as assessed on the same day are shown in Fig. 3. A high HPR total score (> 80%) indicates a very good result, while a low score (< 40%) indicates a poor result. Linear regression analyses for *E. coli* gave the coefficient 0.1 (CI_{95}: 0.06–0.14), and *Enterobacteriaceae* 0.1 (CI_{95}: 0.07–0.13) when the HPR scale was reversed as shown in Fig. 3. This means that for each percentage of poorer HPR-score, *Enterobacteriaceae* and *E. coli* contamination of carcasses increased by 0.1 log CFU/cm^2. The models were highly significant (F-statistics: 28.1, 18 DF, $p = 0.00005$ and 38.99, 18 DF, $p = 0.000007$). For the *Enterobacteriaceae* regression model, R^2 was 0.68 (0.69 for cattle and 0.62 for sheep), and 0.61 for *E. coli* (0.62 for cattle and 0.60 for sheep).

4. Discussion

This study describes slaughter hygiene in 20 bovine and ovine European slaughter lines by three approaches: a standardized study microbial sampling and analysis (baseline); the abattoirs' own routine microbiological testing; and by visual check using the HPR audit.

4.1. Baseline study

To the best of our knowledge, our study reports the methodology and results of the first international baseline study on slaughter hygiene in Europe using a standardized microbial sampling and analysis. Before the introduction of HACCP in the 1990s, a nationwide baseline data collection programme on cattle-carcass hygiene was carried out in USA (Anon, 1994). and Australia. In the EU, however, the microbiological criteria (The Commission of the European Communities, 2005) were established in absence of baseline data. The EU approach was that individual abattoirs should establish their own baseline data and measure their hygiene status against these pre-determined criteria (McEvoy

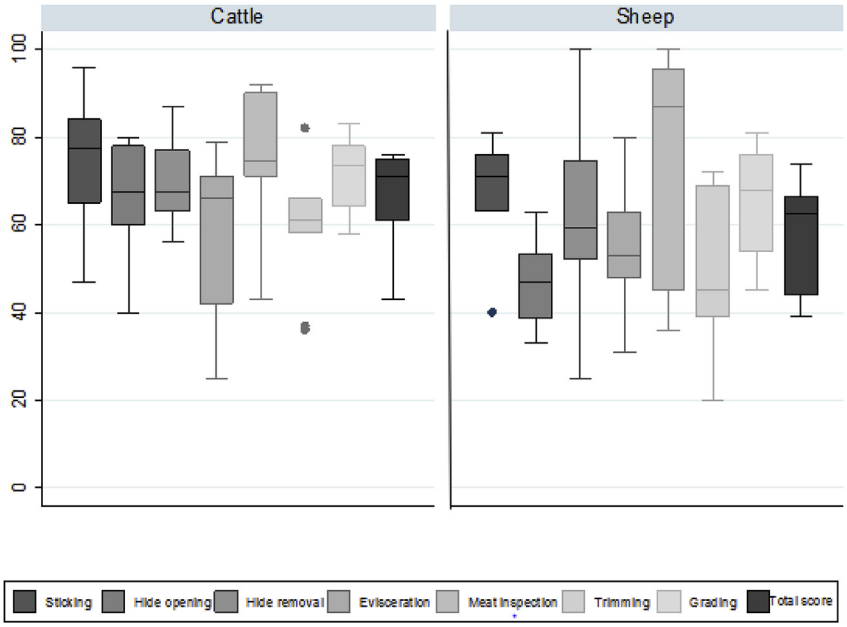

Fig. 2. Box plot for HPR results (%) at the main operations along the slaughter lines combined for cattle (to the left) and sheep (to the right); sticking and bleeding, hide/fleece opening, hide/fleece removal, evisceration, meat inspection, trimming, grading, and weighing, and the total score for cattle to the left and sheep to the right. 100% is perfect slaughter hygiene. The box covers 50% of the results and the line indicates the median and the whiskers indicate upper and lower adjacent values. The dots indicate outside values.

et al., 2004).

In this study, although abattoirs from different European countries generously allowed us to assess their performance for one day, the participating abattoirs should not be considered as representatives from their country, and it was neither the intention nor appropriate to use the study results to benchmark "hygiene levels by country". Nevertheless, this approach could be extended to establish an international benchmarking scheme.

Although the standardized study sampling employed the same sampling technique and analysis, the microbiological results showed considerable variation both within and between abattoirs in carcass contamination. The results also indicated that slaughter hygiene in cattle abattoirs was better than in sheep abattoirs. The differences probably largely reflect systematic factors affecting slaughter hygiene for cattle and sheep (Antic et al., 2010; Reid, Small, Avery, & Buncic, 2002). In Norway, sheep are mostly shorn before slaughter, and carcass contamination of shorn sheep has been found to be less than that of unshorn sheep (Hauge, Wahlgren, Rotterud, & Nesbakken, 2011). In other countries, sheep are slaughtered unshorn or shorn at specific areas of the body, and this may thus affect the hygiene results. Other factors that may affect slaughter hygiene include slaughter line speed, efficient work routines, and number of carcasses per operator. Higher slaughter-line speed often results in more contamination, but well-trained personnel, good routines, and well-functioning equipment may compensate (Hauge et al., 2015; Omer et al., 2015). Additional treatments, such as trimming away visible contamination spots on carcass surfaces with a knife, steam-vacuuming, and removing the outer part of the necks, which were used in four of the sheep slaughter lines included in this study, have shown to have a good decontaminating effect (Hassan, Skjerve, Bergh, & Nesbakken, 2015; Hauge et al., 2011). Washing carcasses with cold water has been shown to have little effect on reducing microbiological contamination of carcasses (Gill, Badoni, & Jones, 1996).

Although the process hygiene criteria have not been established for the standardized sampling procedure that we used, we would expect them to lie between those of the validated Microbiological criteria for process control (Christensen & Olsen, 1999) and the EU's Microbiological criteria for process control. Thus, using a conservative approach and applying the criteria of Christensen and Olsen (1999), the level of faecal contamination was considered acceptable in most abattoirs.

4.2. Abattoirs' routine testing

The abattoirs' sampling regimes resulted in more results below the limit of detection than from the standardized study testing. Several factors may have contributed to this difference, including smaller areas being sampled, different sites on the carcasses being sampled, and that many abattoirs sample cold rather than warm carcasses for their own testing regimes. Lower temperature and desiccation of carcass surfaces result in the levels of bacteria on chilled carcasses being lower than on warm carcasses (Omer et al., 2015). Despite the results obtained by the abattoirs' own testing procedures being mostly below detection limits, they are useful for monitoring carcass hygiene at the individual abattoir level, as trends can be followed. The negative results indicate that the status is good, and should the analyses be sensitive to changes, the results would rise above the detection limit should sporadic contamination problems occur. Such a trend should act as a trigger for the

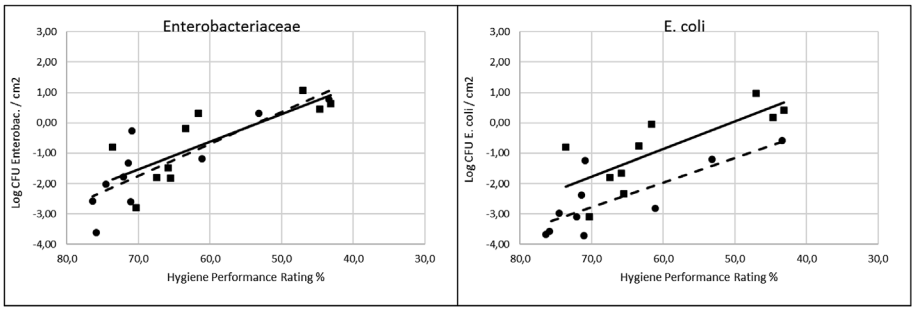

Fig. 3. The relationship between HPR results on the x-axis and microbiological carcass hygiene on the y-axis. The mean of log/cm^2 Enterobacteriaceae (graph to the left) and *E. coli* (to the right) on cattle (dots) and sheep carcasses (squares). The dashed line showed the regression line for cattle and the solid line for sheep. NB: The x-axis is reversed, with low hygienic scores to the right, high to the left of the x-axis.

abattoirs act to investigate any problems, improve their slaughter hygiene, and review the process controls.

Carcass contamination shows both considerable day-to-day variation and carcass-to-carcass variation, and this is exacerbated by other variable factors, such as hot or cold carcass surfaces being sampled, different sampling techniques, varying transport time to the analysing laboratory, as has analysis for different bacteriological markers (Pepperell et al., 2005; Røssvoll et al., 2017). In order to compensate for these variations, samples from representative spot tests of a few carcasses per week should be pooled and it is recommended that the results are analysed statistically in order to recognise trends (EFSA, 2013a, 2013b; The Commission of the European Communities, 2005).

Although many studies have compared different sampling methods and sampling sites (Milios Drosinos, & Zoiopoulos, 2014), few have been able to establish conversion factors or determine new "m" and "M" limits (microbiological load cut-off) according to EU regulation 2073/2005. The "old" EU microbiological performance criteria, as stated in Decision 2001/471/EC (The Commission of the European Communities, 2001), proposed that samples taken by swabbing should have values of 20% of those set for excision samples (Gallina et al., 2015; McEvoy et al., 2004). To the best of our knowledge, only swabbing of cold carcasses with analysis for *E. coli* has been validated (Christensen & Olsen, 1999) and has been widely applied in the international meat market. EFSA compared the Australian monitoring programme (post-chill swabbing) with the European requirements and concluded that an equivalent level of hygiene control can be achieved by calculating and adjusting the M-values (EFSA, 2010), but the values were not presented. FSIS (USA) have standard procedures and guidebooks on the Internet (https://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/). In practice, modifications of protocols from the AOAC International and Association Française de Normalisation (AFNOR) are common, and some contribute to a greater potential for false negative results (Vetter, 2016).

The value to be obtained by comparing between abattoirs has been questioned (Hutchison et al., 2005) as has the usefulness of microbiological analyses to verify a functioning HACCP-system. Nevertheless, despite their pitfalls, microbiological analyses are the only way by which non-visible microbiological contamination can be assessed. Our results demonstrate that microbiological sampling of carcasses can reveal the levels of contamination and reflect the results obtained from the HPR audits. The focus should be directed towards use of appropriate methodology, compliance with standards (De Smedt, 1998; Webster et al., 2006), and competent interpretation of results.

4.3. Hygiene Performance Rating

Audits are usually performed to verify compliance with regulation and proscribed procedures. The HPR approach systematically unmasks defective equipment, poor practices in management and by operators, and other failures that may impact on carcass hygiene and food safety. Our results show that the HPR scores at the abattoirs also reflected the level of carcass contamination, when sampled by third-party samplers. Similar comparisons have previously been conducted; for example, a similar correlation was found between the British HAS and total aerobic counts, but not with coliforms (Hudson, Mead, & Hinton, 1996). However, the association could have been weakened by *ante mortem* assessment being included in HAS. Like HPR, HAS also follows a structured approach and this helps to ensure that the same standard of assessment is applied at every abattoir. The HPR programme includes a direct summary presentation for operators and managers that can be used as a tool to increase competence and awareness of their personal potential impact. Direct dialogue with operators, management, and the meat inspection personnel is crucial, and in this, HPR differs from other auditing protocols that mainly address management.

EFSA has recommended the risk categorization of abattoirs. HPR, supported by microbiological results, might be usefully applied for this task by documentation and benchmarking. Other systems have been used correspondingly. The MHA system (Singleton, 2002, p. 131), is widely used in Australia and provides a standardized monitoring approach that helps to ensure uniformity of processing results across the export meat industry and reflects the need for a zero tolerance of visible contamination of meat with faeces or ingesta (Butler et al., 2003). Other systems that involve categorization of dirty animals presented for slaughter have also been introduced in many European countries (Anon., 2016a; Hauge, Nafstad, Røtterud, & Nesbakken, 2012; Jackson et al., 1999). Dirty animals are more difficult to slaughter hygienically, and categorization and tagging of carcasses contributes to more careful slaughtering and, thus, less carcass contamination (Hauge et al., 2015, 2012).

A significant variation regarding hygiene level, non-validated sampling procedures, corrective actions and compliance with the microbiological criteria was observed. Assuming that our findings are representative to the European meat industry, many companies are obtaining limited compensation for their high hygienic standards. Instead, stimulus for improvement vanish and access to the market is achieved by companies that should actually be encouraged to improve. This does not support fair competition, and those companies that have obtained good level of hygiene are not economically rewarded appropriately. Due to companies' lack of transparency and non-compliance with regulations (e.g. hygienic), they can hide behind a more formal, state-controlled system (Beck, 1988). In consequence, "organized irresponsibility" occurs (Albersmeier, Schulze, Jahn, & Spiller, 2009).

5. Conclusion

Most abattoirs in the study obtained microbiological results that were considered acceptable. Nevertheless, the baseline study of slaughter hygiene showed considerable variation between abattoirs, and cattle slaughter lines had better slaughter hygiene than sheep slaughter lines. The correlation between HPR auditing and the standardized study sampling of carcass contamination indicates that HPR could be used as an effective proxy measure towards improving slaughter hygiene.

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