

## DIAGNOSIS AND DETECTION OF SWINE ZONOTIC DISEASES AND PORK HAZARDS

### 08. In the slaughterhouse, how can the first carcasses be more contaminated with *Enterobacteriaceae* and *E. coli* than the last ones?

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#### Abstract

Pork carcasses' direct or indirect contamination by *Enterobacteriaceae* and *E. coli* (hygiene criteria), mainly by bacteria present in intestinal or skin faecal material, can occur at different stages of the slaughter line. In this study it was determined the level of *Enterobacteriaceae* and *E. coli* contamination on the skin of 100 pigs and in the corresponding carcasses. It also was analysed, for each pig, the skin visible level of faecal contamination (VLFC), recorded the holding time in lairage and the slaughter order (beginning or ending). In each animal, sponge swabs were performed on the skin and in the respective carcasses (approximate 1000 cm<sup>2</sup>). A total of 200 samples were microbiologically analyzed according to ISO 21528-2:2004 (*Enterobacteriaceae*) and ISO 16649-2:2001 (*E. coli*).

The achieved results showed that there was no significant correlation ( $p$ -value  $>0,05$ ) between VLFC in the skin's pig and its level of bacteria contamination which could be due to the shower, used before stunning, that maybe had a different effect on the removal of VLFC and bacteria from the skin (that could still adhered to the skin after shower). Increasing holding time in lairage led to a highly significant increasing level of *Enterobacteriaceae* and *E. coli* ( $p$ -value  $<0.001$ ), both on swines' skin and in the respective carcasses. Achieved results also showed that pigs mean time in lairage was significantly higher for pigs slaughtered at the beginning than for those ones slaughtered at the end of the session ( $p < 0.001$ ), which could help to explain why the average level of *Enterobacteriaceae* and *E. coli* on pigs skin's and in carcasses was significantly higher for pigs slaughtered at the beginning than for those slaughtered at the end.

The results allows to underline lairage logistic and showers efficiency before slaughter as important processes that should be efficiently controlled in order to improve hygiene level of pork carcasses.

#### Introduction

According to Commission Regulation 2073/2005 (amended by Commission Regulation (EC) No 1441/2007), *Enterobacteriaceae* count is considered a process hygiene criteria (PHC) used to evaluate faecal contamination (FC) of fresh meat carcasses. It sets an indicative contamination value above which corrective actions are required in order to maintain the hygiene of the process in compliance with food law. Criteria for the mean *Enterobacteriaceae* counts on pigs are: satisfactory, if the daily mean log is  $< 2,0 \log \text{ cfu/cm}^2$ , acceptable, if the daily mean log is between  $2,0 \log \text{ cfu/cm}^2$  and  $3,0 \log \text{ cfu/cm}^2$  and unsatisfactory, if the daily mean log is  $> 3,0 \log \text{ cfu/cm}^2$ .

*Enterobacteriaceae* and *E. coli* are two interchangeable indicators used to specifically address the level of faecal contamination (Barco *et al.*, 2014). Indicator bacteria are considered an interesting target for microbiological analysis in order to obtain information about the hygiene of processes and products. Microbiological criteria can also be used in validation and verification of HACCP procedures and other hygiene control measures. Also, since pathogenic bacteria are infrequently present on carcass surfaces, generic *E.*

*coli* and other *Enterobacteriaceae* are used to indicate the potential presence of enteric pathogens (Lenahan *et al.*, 2009). The fecal contamination of carcasses, by means of direct or cross-contamination processes, mainly from bacteria present in intestinal or skin faecal material, can occur at different stages of the slaughter practice. According to Vivas and Buncic (2004), in cattle the main source of incoming carcass contamination is the skin. In pigs, the effect of the skin faecal contamination on the microbial profile of the carcasses is less direct/clear than in cattle due to the influence of several steps of the slaughter process (e.g. scalding, polishing...) (Blagojevic *et al.*, 2011). Nevertheless some authors have already proven that contamination of carcasses with *Salmonella* sp. can be directly linked to the skin contamination with his bacteria (Davies *et al.*, 1999; Rossel *et al.*, 2009). According to our knowledge, there are no studies that analyses relationship between the counts of indicator organisms and visual faecal contamination on pig carcasses.

Skin faecal contamination can be related to the holding time in lairage and its level of hygiene. About this subject, Delhalle *et al.*, (2008) already referred the importance of use of water during lairage cleaning and a high frequency of lairage disinfection in lower *E. coli* counts in swine carcass.

The main objectives of present study were to evaluate the association between *Enterobacteriaceae* and *E. coli* (as indicators of faecal contamination) on pigs' skin and respective carcasses and try to explain how these indicators may be affected by visible level of faecal contamination (VLFC) in the pig's skin before slaughter, holding time in lairage and slaughter order (beginning or ending).

## Material and methods

From January 2014 to April 2014, several visits (10) were made to an abattoir in the North of Portugal. In each visit pigs (10) were randomly selected (some at the beginning and some at the ending of the slaughter process) and sampled. For each animal samples were collected from the pigs' skin (after the bleeding) and from the external carcass surface (before refrigeration). Surfaces (1000cm<sup>2</sup> area) were sponge-swabbed starting from the hindquarter downward to the forequarter according to the protocol recommended by the Food Standard Agency (FSA).

Each swab was placed in a separate sterilized recipient properly identified and transported under refrigeration conditions to the laboratory within 2 h.

Each swab-sample was simultaneously used for *Enterobacteriaceae* and *E. coli* count. A total of 100 pigs were analysed. In the laboratory, samples were analyzed according to method defined on ISO 21528-2:2004 and ISO 16649-2:2001 respectively to count *Enterobacteriaceae* and *E. coli*.

Additionally, for each sampled pig the following information was collected:

- Lairage holding (hours): Trough the analyses of the Food Chain Information;
- Slaughter moment: beginning or end of the slaughter day;
- Visible fecal contamination level (VLFC) was evaluated after the bleeding stage: In an imaginary way, the external surface of a half carcass was divided in 4 areas, and each one of theses areas was subdivided in other 4 areas that had a value of 0.25 if VLFC was seen. In that way, VLFC ranged between 0 and 4 values.

On both skins and carcasses, *E. coli* and *Enterobacteriaceae* counts were calculated as CFU/cm<sup>2</sup> and after converted into log CFU/cm<sup>2</sup> and used to calculate mean values. Significance of difference between criteria was calculated using Student T-test.

It was performed a Pearson correlation analysis in order to verify the relationship that could be established

between *Enterobacteriaceae*/*E. coli* level in pigs' skin and carcasses as well as between these indicators and holding time in lairage.

## Results

Table 1 resumes data related to lairage holding time and VLFC

**Table 1** - Data related to lairage holding time and VLFC

Parameters	Lairage holding (Hours)	Visual level of fecal contamination
Mean	27 ± 14,99	0,43 ± 0,54
Minimum	3	0
Maximum	41	2,50
N	100	100

Results concerning to *Enterobacteriaceae* and *E. coli* counts on the pigs' skin and carcasses are presented in table 2.

**Table 2** – *Enterobacteriaceae* and *E. coli* counts (log<sub>10</sub> ufc/cm<sup>2</sup>) on the pigs' skin and carcasses

Parameter	<i>Enterobacteriaceae</i>		<i>E. coli</i>	
	Skin	Carcass	Skin	Carcass
Mean	3,27 ± 0,68	-0,10 ± 0,91	3,15 ± 0,63	-0,06 ± 0,96
Minimum	1,19	-2,18	1,24	-2,00
Maximum	4,84	2,10	4,67	2,49
N	100	100	100	100

## Discussion

In this study, the average time in lairage was 27 hours, which is considered an overstated value and it is not in accordance with Regulation (EC) N.º 853/2004, that defines that animals must be slaughtered without undue delay and only when necessary, for welfare reasons, they should be allowed a rest period before slaughter. This result may have been influenced by the sampling day. All samples were collected on Monday, which means that, probably, we have found animals that arrived at the slaughterhouse during all the weekend period, underlining the difficulty to manage the lairage logistic during this period. Maybe to mitigate this excessive time of holding in lairage, it was observed that the pigs with more time in lairage were the ones to be firstly slaughtered. Student t test showed that the average time spent in the lairage of the pigs slaughtered at the beginning of the slaughter day was higher than that the ones slaughtered at the end, being this difference highly significant (p-value <0.001).

In order to compare the level of visible level of faecal contamination (VLFC) in the pigs'skin at slaughter

with the count of hygiene indicator bacteria, *E. coli* and *Enterobacteriaceae*, it was created a classification scale (between 0 to 4) to quantify VLFC. An average level of 0,45 for VLFC was determined revealing, in general, a relatively clean samples. This result could be related to the use of a shower with high-pressure water that pigs suffered just before stunning (and before the VLFC analyse). According to our results, in this slaughterhouse, VLFC in pig's skin should not be used as an indicator of the presence of these indicator bacteria since the statistical results didn't support any significant difference in *E. coli* and *Enterobacteriaceae* count among pigs with different levels of VFC (p-value >0,05). As it was referred before, this could be related to the fact that the VLFC was analysed after the cleaning (shower) step. In this case, it seems that the shower used to clean the pigs before slaughter was effective in the reduction of visual dirtiness but, apparently, not effective in bacteria removal that could still adhered to the skin or, on the other hand, the washing procedure could had lead to a redistribution of bacteria over skin surface. Belluco *et al.* (2015) referred this situation in pig carcasses, mentioned that washing does not effectively reduce microbial contamination.

There are several studies published on the influence of different stages of the slaughterline on the count of *E. coli* and *Enterobacteriaceae* on swine carcasses. But, according to our knowledge, this is the first study that analyses relationship between the counts of indicator organisms and visual faecal contamination on pig carcasses. This gap was previously referred in a literature review done by Barco *et al.*, 2014.

Although it was observed a highly significant (p-value < 0,001) reduction in *Enterobacteriaceae* and *E. coli* counts from pigs 'skin to the carcass, indicating the efficiency of the slaughter process in the reduction of these indicators, it was also demonstrated a highly significant (p-value < 0,001) positive correlation between these two results: higher counts of *Enterobacteriaceae* and *E. coli* in pigs 'skin are related to increases in counts in the respective carcass. Additionally, the achieved results showed that increasing time in lairage holding leads to an increasing level of *Enterobacteriaceae* and *E. coli* both in swine skin (p-value <0.001) and in the respective carcass (p-value <0.001), been these relationships highly significant. Since, in this study, pigs slaughtered at the beginning had a mean time in lairage significantly higher than those slaughtered at the end of the session (p<0.001), that could help to explain why the average level of *Enterobacteriaceae* and *E. coli* in the first carcasses was significantly higher than in those processed at the end. In a slaughterhouse, this should be considered an unusual result. As it was referred by Greig *et al.* (2005), the usual is the last carcasses be more contaminated that the first ones due to the crescent contamination (instruments, equipment...) along the slaughter process. These result highlights the importance of slaughter "clean" pigs (as it is referred in European Regulation 853/2004) or the use of effective cleaning measures before slaughter as well the reduction of holding time in lairage as important measures to improve hygienic level of carcasses.

## Conclusions

Results from this study underpin the influence of skin faecal contamination and lairage holding time on the level of *Enterobacteriaceae* and *E. coli* in carcasses. Both indicators led to the same conclusions. Since pig's skin proved to be an important source of carcass contamination, further studies should be derived in order to better understand this subject and analyse effective hygienic practices to control it. According to our knowledge, this is the first study that analyses relationship between the counts of indicator organisms and visual faecal contamination on pig carcasses. In this study, VLFC wasn't considered an good indicator of the presence *E. coli* and *Enterobacteriaceae* . Maybe in next studies we should try to classify VLFC before the cleaning step.

According to European Regulation CE) N.º 2073/2005, when the results of testing against this hygiene criteria are unsatisfactory, improvements in slaughter hygiene and review of process controls must be implemented. Our results indicate that these improvements should advantageously include corrective actions to increase hygiene level of the pigs' skin before slaughter and reduce holding time in lairage.

The present study aimed to provide useful information for the implementation of good hygienic practices during the pig slaughter process.

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