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Use of 16S rDNA Metagenetics and classical Microbiology to Assess the bacterial superficial Contamination Patterns in Bovines Classically Slaughtered or following the Halal Ritual

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INTRODUCTION

In several European countries, two cattle slaughtering protocols exist: the classical method, that encompasses a stunning step before the sticking procedure, and the halal method, combining the stunning and the sticking in one single step. The main difference lies in the fact that, in the halal protocol, a single cut with a sharp knife is practiced directly on live cattle, instead of two cutting steps with two different knives for the sticking in the classical slaughtering technique. The unique section in the halal technique results generally in the cross section of trachea and esophagus of cattle.

OBJECTIVES

The aim of this study was to seek if the two slaughtering techniques were similar regarding the superficial microbial contamination of carcasses, swabbed between 2 and 4 hours after the killing step.

MATERIALS AND METHODS

Abattoir

Belgian Cattle slaughterhouse:

- east of Belgium
- practicing both slaughtering methods
- separated non-rotating contention box for Halal slaughtering
- ligation of esophagus in both cases

Sampling

Period of sampling: Augustus 2013

2 days of sampling

20 swabbed carcasses (10 Classically slaughtered and 10 Halal-slaughtered)

Sex of animals: 19 male and 1 Heifer

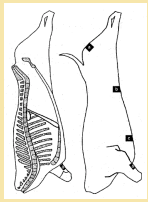
Swabbing

Wet-cotton swabbing method

2 to 4 hours after slaughtering

2 zones:

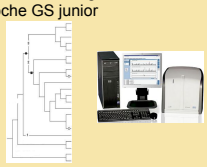
- 1,600 cm² ("legal" zone)
- neck area (200 cm2) close to the bleeding cut



Methods

Mesophilic Total Viable counts (TVC) at 30 °C **+ counting of *Enterobacteriaceae*** (VRBG 30 °C)

Metagenetic analysis targeting the V1-V3 region of the 16S rDNA was performed using the Roche GS junior



Statistics

TVC and *Enterobacteriaceae*:

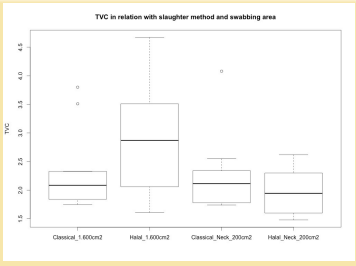
- non-parametrics statistics (Mann-Whitney)

Metagenetics

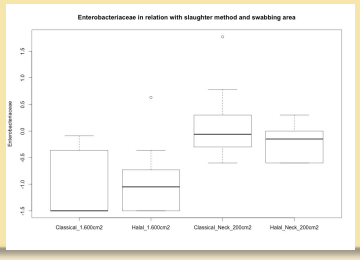
- percentage of reads for each OTU
- conversion in cfu / g (in relation with TVC results)
- Newcombe-Wilson Stat (Stamp software)

RESULTS (I)

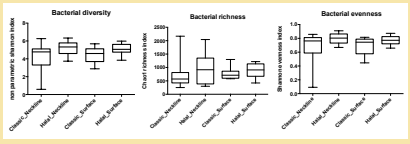
TVC & *Enterobacteriaceae* (log UFC / cm²)

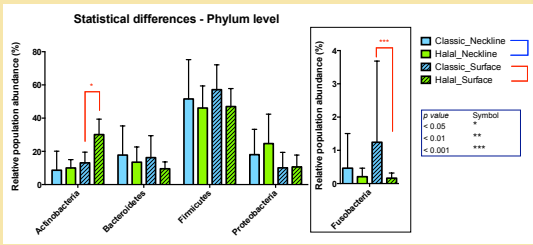


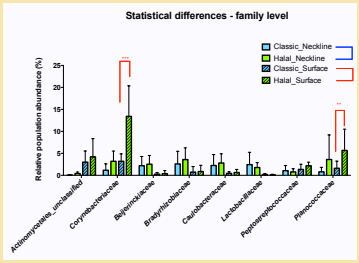
no significant difference observed

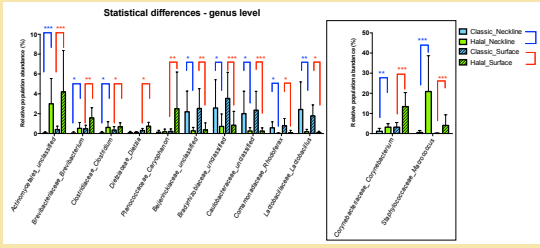


Metagenetics









RESULTS (II)

Classical microbiology, non significant differences, but:

- higher contamination level in the "legal" zone (1,600 cm2),
- and lower level for the neck arae (200 cm2)

in the "Halal" group compared to the "Classic" group

Metagenetics – "Halal" vs "Classic"':

- Phylum level
  - differences in the "legal" zone
  - ↗ *Actinobacteria* & ↘ *Fusobacteria*
- Family level ↗ in the "legal" zone for
  - *Corynebacteriaceae, Planococcaceae, Aerococcaceae, Brevibacteriaceae and Clostridiaceae*
- Family level ↗ in the "neck" zone for
  - *Aerococcaceae and Clostridiaceae*
- Genus level ↗ in all the zones for
  - *Brevibacterium, Clostridium, Corynebacterium & Macrococcus*
- Genus level ↘ in all the zones for
  - *Beijerinckiaceae, Bradyrhizobiaceae,* and *Caulobacteriaceae* genera, *Rhodoferax & Lactobacillus*

DISCUSSION & CONCLUSIONS

The metagenetics reveals different patterns of contamination between swabbing areas and slaughtering techniques

The slaughtering method does not influence the superficial microbiological pattern in terms of specific microbiological markers of the digestive or respiratory tract.

The legal zone of swabbing reflects the hygienic conditions of slaughtering. Further studies are needed to correlate the superficial contamination with hygienic practices