

# The effects of a non-intervention HACCP implementation on process hygiene indicators on bovine and porcine carcasses

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## Summary

Four sites on each of 720 dressed carcasses (360 bovine and 360 porcine) were sampled (2,880 samples in total) in a single commercial abattoir slaughtering cattle and pigs using two separate slaughterlines. The carcasses were sampled before HACCP (pre-HACCP; 960 samples) and after HACCP implementation (post-HACCP; 1,920 samples) and Total Viable Count (TVC), Enterobacteriaceae count (EC) and *Salmonella* spp. prevalence were determined. During the pre-HACCP period, mean TVC levels on four tested sites varied on bovine carcasses between 3.03 and 4.19 log<sub>10</sub> cfu/cm<sup>2</sup> and on porcine carcasses between 3.73 and 3.99 log<sub>10</sub> cfu/cm<sup>2</sup>. During the post-HACCP period, TVC levels on all tested sites on carcasses were further significantly reduced, by 0.33-1.64 log and 1.13-2.04 log on bovine and porcine carcasses, respectively, compared to the pre-HACCP period. Both the EC occurrence in samples and EC levels in EC-positive samples somewhat decreased during post-HACCP as compared to pre-HACCP period, but the reductions were not statistically significant due to large proportion of EC-negative samples and very low counts in EC-positive samples. *Salmonella* spp. was not detected in any of bovine or porcine carcass samples, regardless of whether they were taken pre- or post-HACCP. Overall, the process-hygiene-improving effects of non-intervention HACCP have been proven through reduction of TVC on carcasses, but could not be verified in the present study through similar reductions in EC and/or *Salmonella*, because of their low levels and/or absence.

**Key words:** Non-intervention HACCP, carcass microbiology, process hygiene

## 1. Introduction

Sources of microbiological contamination of carcass meat during slaughter and dressing of animals include the faeces, the hide, intestinal contents, lymph nodes, processing equipment, water, air and humans (Sofos, 2005). Strict and adequate maintenance of good hygiene practices at each operational step along the slaughter line (i.e. process hygiene) is of crucial importance for the prevention

or minimization of microbial contamination of final carcasses, so to ensure meat safety and protect public health (Zweifel, Baltzer & Stephan, 2005; Tergney & Bolton, 2006). Presently, this is achieved through implementation of meat safety programs based on Hazard Analysis and Critical Control Points (HACCP) plans. The Critical Control Points (CCPs) may vary depending on the slaughterline technologies, but generic CCPs in abat-

toirs include acceptance of animals (i.e. defined cleanliness score), de-hiding and evisceration steps (Norrung & Buncic, 2008).

Two main HACCP types are: the so-called "intervention" HACCP and "non-intervention" HACCP. The former is used in the USA and mandatorily includes application of decontamination treatments of carcasses. The latter is used

**Table 1** Microbial process hygiene indicators on bovine and porcine carcasses  
**Tablica 1.** Indikatori mikrobiološke procesne higijene na govedim trupovima

Bovine carcass sites (number of samples) Mjesta na govedem trupu (broj uzoraka)	Total viable count of bacteria (TVC; mean log <sub>10</sub> cfu/cm <sup>2</sup> ± SD)		Enterobacteriaceae count / Broj enterobakterija (EC; mean log <sub>10</sub> cfu/cm <sup>2</sup> ± SD)*		<i>Salmonella</i> spp.		
	Pre-HACCP period (months - 5 to 0) Pre-HACCP period (mjeseci - 5 do 0)	Initial post-HACCP period (months 0 to 5) Početni post-HACCP period (mjeseci 0 do 5)	Pre-HACCP period (months - 5 to 0) Pre-HACCP period (mjeseci - 5 do 0)	Advanced post-HACCP period (months 6 to 10) Napredni post-HACCP period (mjeseci 6 do 10)			
Rump (120 per period) But (120 po periodu)	3.03±0.62A	2.54±0.58B	2.7±0.37C	0.20±0.45D	0.00±0.00D	0.16±0.37D	ND
Flank (120 per period) Potrbušina (120 po periodu)	4.19±0.34A	2.68±0.82B	2.45±0.33B	0.19±0.97D	0.78±0.17D	0.29±0.41D	ND
Brisket (120 per period) Grudi (120 po periodu)	3.77±0.49A	2.56±1.00B	2.86±0.40B	0.32±0.71D	0.00±0.00D	0.08±0.18D	ND
Neck (120 per period) Vrat (120 po periodu)	3.32±0.63A	2.12±0.62B	2.20±0.38C	0.28±0.62D	0.00±0.00D	0.00±0.00D	ND

SD Standard deviation; \* Calculated only for bovine carcasses. Values in the same row are significantly different (p<0.05).

SD Standardna devijacija; \*Izračunata samo za govede. Vrijednosti u istom redu su značajno različite (p<0.05).

in the EU and relies only on effective process hygiene i.e. does not include carcass decontamination treatment.

According to the current EU legislation (Regulation (EC) 2073/2005 and 1441/2007), the process hygiene criteria for abattoirs specify acceptable and unacceptable levels of indicator organisms (Total Viable Count, TVC; and *Enterobacteriaceae* count, EC) on carcasses, at the end of slaughterline but before their chilling. However, although microbiological testing of carcasses based

on bovine carcasses is not required, process hygiene on govedim trupovima

Bovine carcass sites (number of samples) Mjesta na govedem trupu (broj uzoraka)	Total viable count of bacteria (TVC; mean log <sub>10</sub> cfu/cm <sup>2</sup> ± SD)		Enterobacteriaceae count / Broj enterobakterija (EC; mean log <sub>10</sub> cfu/cm <sup>2</sup> ± SD)*		<i>Salmonella</i> spp.		
	Pre-HACCP period (months - 5 to 0) Pre-HACCP period (mjeseci - 5 do 0)	Initial post-HACCP period (months 0 to 5) Početni post-HACCP period (mjeseci 0 do 5)	Pre-HACCP period (months - 5 to 0) Pre-HACCP period (mjeseci - 5 do 0)	Advanced post-HACCP period (months 6 to 10) Napredni post-HACCP period (mjeseci 6 do 10)			
Rump (120 per period) But (120 po periodu)	3.03±0.62A	2.54±0.58B	2.7±0.37C	0.20±0.45D	0.00±0.00D	0.16±0.37D	ND
Flank (120 per period) Potrbušina (120 po periodu)	4.19±0.34A	2.68±0.82B	2.45±0.33B	0.19±0.97D	0.78±0.17D	0.29±0.41D	ND
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on bovine carcasses is not required, process hygiene on govedim trupovima is not required. Values in the same row are significantly different (p<0.05). SD Standardna devijacija; \*Izračunata samo za govede. Vrijednosti u istom redu su značajno različite (p<0.05).

use criteria enables the detection of process hygiene changes in the total carcass microbiota, it does not provide sufficient evidence to elucidate the origin and/or the extent of the microbial contamination by pathogenic organisms. Furthermore, bacterial pathogens usually present in low numbers on carcasses and their presence is not necessarily accompanied by an increase in total bacterial counts (Warriner, Aldsworth, Kaur & 2002). In addition to TVC and EC the EU process hygiene criteria specify unacceptable prevalence

of the foodborne pathogen *Salmonella* spp. However, the overall probability of detection of any bacterial pathogen (including *Salmonella*) on carcasses is not determined only by the process hygiene, but also by the pre-slaughter factors determining the occurrence of the pathogen in incoming animals.

Verification that a HACCP-based system works effectively involves comparing the performance parameters of each operator with their own parameters obtained in different periods;

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with the parameters of other operators; and with the parameters from national and international baselines/databases (Buncic, 2006). Nevertheless, the question of whether, and by how much, the implementation of non-intervention HACCP (which has no bactericidal step) in abattoirs actually improves microbial status of carcasses is often debated. To answer such a question directly, the same microbiological parameters of the hygienic status of carcasses need to be determined (by the same methods) before and after HACCP implementation, and then compared. In the USA, some reports indicate that microbiological safety of meat improved after HACCP implementation, because *Salmonella* occurrence on cattle, pig and poultry carcasses decreased post-HACCP as compared with the pre-HACCP situation (Rose et al., 2002; FSIS, 2004). In the EU, Cenci-Goga et al. (2005) reported that HACCP application contributed to improve the food safety of meals served in a restaurant in Italy. However, data obtained at abattoirs through systematic, standardized microbiological testing of carcasses before HACCP implementation are lacking in the EU; only data related to post-HACCP-implementation carcass testing are readily available for the EU countries. The reasons for that include the fact that official methodology of carcass sampling and testing was either not available or not used within the EU before it was described and prescribed by the EU legislation making HACCP mandatory since 1<sup>st</sup> January 2006 (EU "Hygienic Package"; Regulation 853/2004/EC).

However, mandatory HACCP implementation in the meat industry in Serbia started on 1<sup>st</sup> January 2009 (Veterinary Law; OJ RS 91/05), which was a 3-year delay compared to the EU. During this delay period, the official EU carcass testing methodology was available, so it could be used voluntarily in Serbia. Therefore, the aim of the present study was to evaluate the effectiveness of HACCP through comparison of process hygiene parameters, determined

by the official EU methodology, before and after implementation of non-intervention HACCP system. To minimize the effects of other co-interfering variable factors (e.g. incoming animals, technology- and staff-related) on the pre-HACCP vs. post-HACCP comparison, the data was obtained on a single abattoir operating separate slaughterlines for cattle and pigs.

## 2. Materials and methods

### 2.1. Pre-HACCP and post-HACCP sampling periods

Four samples from each of 720 sampled dressed carcasses (360 bovine and 360 porcine) were collected (2,880 samples in total) in a single commercial abattoir slaughtering cattle and pigs at two separate slaughterlines. The samples were collected over two periods, before HACCP implementation and after HACCP implementation; the starting day of HACCP implementation was marked as "0" time. Firstly, 960 samples were collected during the 5-month period preceding the HACCP implementation (pre-HACCP period; months -5 to 0). Secondly, 1,920 samples were collected during the 10-month period following the HACCP implementation (post-HACCP period). For better pre-HACCP versus post-HACCP comparison purposes, the post-HACCP period was divided into two sub-periods: initial post-HACCP period (960 samples collected during months 0 to 5) and advanced post-HACCP period (960 samples collected during months 6 to 10).

### 2.2. Sampling of carcasses

Carcasses were sampled at the end of slaughterline: after washing, but before chilling. The official EU carcass swabbing methods, as well as referent ISO standard were used (Commission Decision 2001/471/2001; ISO 17604:2003).

Sampling for general microflora indicators (Total Viable Count and Enterobacteriaceae). In the case of bovine carcasses, the following four sites were sampled: rump, flank, brisket and neck.

In the case of porcine carcasses, the following four sites were sampled: ham, back, belly and jowl. A sterile metal template (delineating 100cm<sup>2</sup>; sanitized after each sample using 70% ethanol) was placed on pre-determined carcass site and the area was swabbed (stroking 10 times vertically, horizontally and diagonally). First, each area was swabbed using wet swab moistened by dipping in Maximum Recovery Diluent (MRD; 0.85% NaCl + 0.1% peptone; Oxoid, UK). Then, the same area was swabbed using a dry swab. From each sampling site, wet and dry swabs were placed together in a stomacher bag and transported to the laboratory within 3-4 hours, in a chill box at 0-4°C.

Sampling for *Salmonella* spp. On each carcass, the lateral brisket site was sampled. The abrasive sponge (10x10 cm) was exposed to the UV for 10 min and wrapped in aluminum foil. Immediately before taking the samples, the unwrapped sponge was placed into stomacher bag (19x30 cm; Nasco, Whirl-pak, USA), wetted with 10 ml MRD and then manually massaged from outside the bag to moisten it thoroughly. A sterile metal template delineating 100 cm<sup>2</sup> was placed on the sampled site. Using sterile gloves, the sponge was carefully removed from the stomacher bag and the delineated area was swabbed (stroking 10 times vertically, horizontally and diagonally). Afterward, the sponge was placed back in the stomacher bag and transported to the laboratory within 3-4 hours, in a chill box at 0-4°C.

### 2.3. Microbiological methods

Total Viable Count of bacteria (TVC). The ISO 4833:2003 method was used to determine TVC. MRD (90 ml) was added to each stomacher bag containing the wet-dry swabs; the bag was then manually massaged from the outside for 2 min to obtain the homogenate. The homogenate obtained was serially diluted, and one ml volumes were spread-plated on duplicate plates of Plate Count Agar (PCA; Merck, Ger-

many). The plates were incubated aerobically for 72 h at 30°C.

Enterobacteriaceae count (EC). The ISO 21528-2:2004 method was used to determine EC. The homogenate (described for TVC) was serially diluted, and one ml volumes were spread-plated on duplicate plates of Violet Red Bile Glucose Agar (VRBG; Merck, Germany). The plates were incubated for 24 h at 37°C, and the presumptive colonies were subcultured onto PCA agar and confirmed as *Enterobacteriaceae* biochemically (API 20E; Biomerieux, France).

*Salmonella* spp. detection. The procedure essentially followed the ISO 6579:2002 method. MRD (90 ml) was added to each stomacher bag containing the wet-dry swabs; the bag was then manually massaged from the outside for 2 min to obtain the homogenate. The homogenate was incubated for 18 h at 37°C. A 0.1 ml volume was inoculated first in selective enrichment medium: Rappaport-Vassiliadis broth with soya (RVS; Merck, Germany) and incubated for 24 h at 42°C. From the same homogenate, a one ml volume was also inoculated in a second selective enrichment medium: Müller-Kauffmann tetrathionate/novobiocin broth (MKTn; Merck, Germany) and incubated for 24 h at 37°C. From each broth culture, two solid selective media were streak-inoculated: Xylose Lysine Deoxycholate agar (XLD; Merck, Germany) and Brilliant green agar (BGA; Merck, Germany). The XLD and BGA plates were incubated for 24 h at 37°C, and up to five presumptive colonies were subcultured onto PCA agar and confirmed as *Salmonella* spp. biochemically (API 20E; Biomerieux, France).

### 2.4. Analysis of results

The results were expressed either as present/absent on 100 cm<sup>2</sup> carcass surface. The detection limit for both TVC and EC was 5.0x10<sup>2</sup> cfu per cm<sup>2</sup> carcass surface. For both TVC and EC, the cfu/cm<sup>2</sup> values were converted to log<sub>10</sub> cfu/cm<sup>2</sup> before calculation of mean values,

standard deviation and significance of difference between means using ANOVA (SPSS package).

## 3. Results and discussion

### 3.1 Process hygiene indicators on bovine carcasses

#### Total Viable Count of bacteria (TVC)

In the abattoir process hygiene context, TVC is considered as indicator of general microbial contamination. During the pre-HACCP period (months -5 to 0; the latter is the start of HACCP implementation), mean ACC levels on tested sites on bovine carcasses varied between 3.03 and 4.19 log<sub>10</sub> cfu/cm<sup>2</sup> (Table 1). These mean TVCs were higher than those reported (unrelated to the start of HACCP implementation) for bovine carcass in some other studies: 3.63 (Zweifel et al., 2008), 3.5 (McEvoy, Sheridan, Blair & McDowell, 2004), 3.1 (Phillips, Sumner, Alexander & Dutton, 2001), 2.83 (Hutchison et al. 2005, 2007) or 1.8 log<sub>10</sub> cfu cm<sup>2</sup> (Sumner, Petrenas, Dean, Dowsett, West & Wiering, 2003). With respect to different sites on carcasses, the mean TVC levels were in decreasing order: flank>brisket>neck>rump (Table 1). Zweifel, Fischer & Stephan (2008) reported the highest level of microbial contamination in the neck and brisket sites (3.83 and 3.46 log<sub>10</sub> cfu cm<sup>2</sup>, respectively). The differences between the present and the quoted studies, in respect to both levels and distribution of TVC on bovine carcasses, can be attributed to a number of factors that probably differed between the studies; particularly to the microbial levels/distribution in/on incoming cattle and the slaughter-dressing practices.

During the initial post-HACCP period (months 0 to 5), significant decrease (by 0.49 log) in TVC on rump was observed (Table 1). Although TVC decreased on other sampled carcass sites during the initial post-HACCP period as well, the decrease was not statistically significant. During the advanced post-HACCP period (months 6 to 10), TVC levels on all four carcass sites were

further significantly reduced (by 0.33-1.64 log; depending on site) compared to the pre-HACCP period (Table 1). In that period, the highest mean TVC was observed on brisket, which is different compared to the pre-HACCP period - when flank was the most contaminated site.

#### Enterobacteriaceae count (EC)

In the abattoir process hygiene context, EC is considered as indicator of microbial contamination of faecal origin. In the present study, *Enterobacteriaceae* were detected in only 16.3% of bovine samples. In other studies, the reported overall proportion of bovine carcasses positive for *Enterobacteriaceae* were 22% (Zweifel et al., 2008) or 23.4% (Hutchison et al., 2005, 2007). In the present study, in positive bovine carcass samples during pre-HACCP period, the mean EC levels varied between 0.19 and 0.32 log<sub>10</sub> cfu/cm<sup>2</sup>, which was much lower than corresponding TVC levels (Table 1), whilst Hutchison et al. (2005, 2007) reported an average of 0.69 log<sub>10</sub> cfu/cm<sup>2</sup>. In the present study, the EC levels differed little between the four carcass site, in decreasing order brisket>neck>rump>flank, but the differences were statistically insignificant.

During the initial and the advanced post-HACCP periods, the levels of *Enterobacteriaceae* in positive samples somewhat decreased, compared to pre-HACCP situation (Table 1). However, the reductions were not statistically significant due to large proportion of EC-negative samples and very low counts in EC-positive samples.

#### *Salmonella* spp.

*Salmonella* spp. was not detected in any of bovine carcass samples, regardless whether they were taken pre- or post-HACCP (Table 1). This was somewhat surprising because the pathogen was found on bovine carcasses in some other studies, with prevalence e.g. 7.6% (McEvoy, Doherty, Sheridan, Blair & McDowell, 2003) or even up to 50.2% (Brichta-Harhay, Guerini, Arthur, Bosile-

**Table 2** Microbial process hygiene indicators on porcine carcasses  
**Tablica 2.** Indikatori mikrobiološke procesne higijene na trupovima svinja

Porcine carcass sites (number of samples) Trupovi svinja (broj uzoraka)	Total viable count of bacteria / Ukupan broj bakterija (TVC; mean log <sub>10</sub> cfu/cm <sup>2</sup> ±SD)			Enterobacteriaceae count / Broj Enterobakterija (EC; mean log <sub>10</sub> cfu/cm <sup>2</sup> ±SD)*			Salmonella spp.
	Pre-HACCP period (months – 5 to 0)	Initial post-HACCP period (months 0 to 5)	Advanced post-HACCP period (months 6 to 10)	Pre-HACCP period (months – 5 to 0)	Initial post-HACCP period (months 0 to 5)	Advanced post-HACCP period (months 6 to 10)	All three periods
	Pre-HACCP period (mjeseci – 5 do 0)	Početni poslije-HACCP period (mjeseci – 0 do 5)	Napredni poslije-HACCP period (mjeseci – 6 do 10)	Pre-HACCP period (mjeseci – 5 do 0)	Početni poslije-HACCP period (mjeseci – 0 do 5)	Napredni poslije-HACCP period (mjeseci – 6 do 10)	Sva tri perioda
Ham (120 per period) But (120 po periodu)	3.98±0.61A	2.77±0.33B	1.94±0.12C	0.91±0.83D	0.41±0.90D	0.00±0.00D	ND
Back (120 per period) Leđa (120 po periodu)	3.73±0.87A	1.68±0.24B	2.19±0.24B	0.00±0.00D	0.00±0.00D	0.08±0.18D	ND
Belly (120 per period) Potrbušina (120 po periodu)	3.92±0.82A	2.36±0.41B	2.52±0.32B	0.66±0.94D	0.05±0.11D	0.07±0.18D	ND
Jowl (120 per period) Obrazine (120 po periodu)	3.99±0.62A	3.09±0.32B	2.86±0.55B	1.23±0.79D	0.05±0.11D	0.08±0.18D	ND

SD Standard deviation; \* Calculated only from positive samples; ND Not detected; A, B, C, D Means with different letters in the same row are significantly different (p<0.05);

SD Standardna devijacija; \*Izračunata samo od pozitivnih uzoraka; ND Nije detektiran; A, B, C, D Srednje vrijednosti s različitim slovima u istom redu su značajno različite (p<0.05)

vac, Kalchayanand, Shackelford, Wheeler & Koohmaria, 2008). No direct explanation for the lack of *Salmonella* on bovine carcasses in the present study can be offered, but contributing factors may include a possible very low prevalence in the cattle population and/or very low counts of the pathogen on carcasses (i.e. below limit of detection).

### 3.2 Process hygiene indicators on porcine carcasses

#### Total Viable Count of bacteria (TVC)

During the pre-HACCP period, mean TVC levels on tested sites of porcine

carcasses varied between 3.73 and 3.99 log<sub>10</sub> cfu/cm<sup>2</sup> (Table 2). Different values have been reported for porcine carcasses in other studies: 4.5-4.7 (Bolton, Pearce, Sheridan, Blair, McDowell & Harrington, 2002), 4.48 (Hutchison et al. (2005, 2007) or 3.66 log<sub>10</sub> cfu cm<sup>2</sup> (O'Brien, Lenahan, Sweeney & Sheridan, 2007). With respect to different sites on carcasses in the present study, the mean TVC levels were in decreasing order jowl>ham>belly>back, but the differences were statistically insignificant. In contrast, Zweifel et al. (2008) found the highest contamination level

in the back region. As indicated above, between-studies differences in respect to both levels and distribution of TVC on porcine carcasses, can be attributed to a number of factors variable factors including the microbial levels/distribution in/on incoming pigs and the slaughter-dressing practices.

During the initial post-HACCP period (months 0 to 5), marked and significant decreases in TVC on all four carcass sites (by 0.9-2.05 log; depending on site) were observed, compared to the pre-HACCP period (Table 2). During the

advanced post-HACCP period (months 6 to 10), TVC levels on all four carcass sites were also significantly reduced (by 1.13-2.04 log; depending on site) compared to the pre-HACCP period (Table 2).

#### Enterobacteriaceae count (EC)

In the present study, *Enterobacteriaceae* were detected in only 11.7% of porcine samples. In other studies, reported overall proportion of porcine carcasses with relatively low incidence and at low levels, which prohibits accurate analysis and interpretation of the results (Mc Evoy et al., 2004). Nevertheless, determination of *Enterobacteriaceae* still can be considered as essential part of slaughter hygiene verification, as it can provide important information on faecal contamination of carcasses (Zweifel et al., 2008). Although high levels of TVC and/or *Enterobacteriaceae* may not always correlate with the presence of pathogenic bacteria on carcasses, they indicate that increased microbial meat safety risks may exist, which requires ensuring stricter process hygiene. The usefulness of these criteria is further increased when abattoirs establish their own related databases and match them with process-specific circumstances (Pearce, Bolton, Sheridan, McDowell, Blair & Harrington, 2004; Vanne, Karwoski, Karppinen & Sjoberg, 1996).

During the initial and the advanced post-HACCP periods, the levels of *Enterobacteriaceae* in positive samples somewhat decreased, compared to the pre-HACCP situation (Table 2). However, the decreases were not statistically significant due to the large proportion of EC-negative samples and very low counts in EC-positive samples.

#### Salmonella spp.

*Salmonella* spp. was not detected in any of the porcine carcass samples, regardless whether they were taken pre- or post-HACCP (Table 1). This was somewhat surprising because the pathogen was found on porcine carcasses in some other studies, with prevalences of 6-7% (Bolton et al., 2002) or up to 13% (Bradshaw & Leonard, 2006). Similarly to bovine carcasses, no direct explanation for the lack of *Salmonella* on porcine carcasses in the present study can be offered, but contributing factors may include possible very low prevalence in the pig population and/

or very low counts of the pathogen on carcasses (i.e. below limit of detection).

### 3.3 Overall impact of HACCP implementation on process hygiene

With respect to choice of the microbial process hygiene indicators, TVC is considered as the simplest and most reliable method of monitoring carcass hygiene (Mackey & Roberts, 1993), because enteric bacteria occur on carcasses with relatively low incidence and at low levels, which prohibits accurate analysis and interpretation of the results (Mc Evoy et al., 2004). Nevertheless, determination of *Enterobacteriaceae* still can be considered as essential part of slaughter hygiene verification, as it can provide important information on faecal contamination of carcasses (Zweifel et al., 2008). Although high levels of TVC and/or *Enterobacteriaceae* may not always correlate with the presence of pathogenic bacteria on carcasses, they indicate that increased microbial meat safety risks may exist, which requires ensuring stricter process hygiene. The usefulness of these criteria is further increased when abattoirs establish their own related databases and match them with process-specific circumstances (Pearce, Bolton, Sheridan, McDowell, Blair & Harrington, 2004; Vanne, Karwoski, Karppinen & Sjoberg, 1996). Generally, optimal hygienic practices and effective process hygiene management are crucial for successful workings of non-intervention HACCP system (Bolton, Doherty and Sheridan, 2001).

In the present study, mean values of all three process hygiene indicators (TVC and EC levels, as well as *Salmonella* occurrence) on both bovine and porcine carcasses did not exceed the corresponding unacceptable ranges given in the EU process hygiene criteria (Regulations 2073/2005/EC and 1441/2007/EC); neither pre-HACCP nor post-HACCP implementation. Nevertheless, the post-HACCP decrease of TVC on carcasses clearly indicated that implementation of the non-intervention HACCP system

significantly improved the process hygiene of both cattle- and pig- slaughterlines in the abattoir. This was related mainly to implemented improvements in the slaughterline practices, particularly including workers' personal hygiene, hygiene of critical operations, and the use of a hygiene monitoring system at each CCP. The process-hygiene-improving effects of HACCP implementation, proven through reduction of TVC on carcasses, could not be verified in the present study also through similar reductions in EC and/or *Salmonella*, because of low levels and absence, respectively, of the latter two microbial indicators on carcasses. Nevertheless, it can be expected that the microbiologically beneficial effects of non-intervention HACCP are not limited only to TVC, but they would have included also enteric organisms, including pathogens, if they had been present at higher levels on carcasses in the present study. This presumption is based on results from other studies confirming that implementation of HACCP-based food safety system produced significant reductions in foodborne pathogens e.g. *Salmonella* on carcasses at abattoirs (Rose et al., 2002; FSIS, 2004) or *S. aureus* and *B. cereus* in meals in restaurants (Cenci-Goga et al., 2005). Overall, further research is necessary to optimize approaches and methods for verification of HACCP system at abattoirs through microbiological process hygiene indicators in situations where levels/occurrence of either *Enterobacteriaceae* or *Salmonella* (or both) are very low.

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## Učinak primjene "neinterventnog" HACCP-sistema na indikatore procesne higijene na govedim i svinjskim trupovima

### Sažetak

Sa svakog od 720 obrađenih trupova (360 govedih i 360 svinjskih) uzeti su uzorci s četiri mjesta na trupu (ukupno 2,880 uzoraka), u jednoj komercijalnoj klaonici za klanje goveda i svinja, s dvije odvojene linije. Uzorci s trupova su uzeti prije primjene HACCP sistema (pre-HACCP; 960 uzoraka), nakon primjene HACCP sistema (post-HACCP; 1,920 uzoraka) i utvrđivan je ukupan broj bakterija (UBB), broj *Enterobacteriaceae* (EC) i prevalencija *Salmonella* spp. Tijekom pre-HACCP perioda, srednji ukupan broj bakterija, sa četiri testirana mjesta na trupu se razlikovao na govedim trupovima između 3.03 and 4.19 log<sub>10</sub> cfu/cm<sup>2</sup> i na trupovima svinja između 3.73 i 3.99 log<sub>10</sub> cfu/cm<sup>2</sup>. Tijekom perioda post-HACCP-a, ukupan broj bakterija sa svih testiranih mjesta na trupovima je bio značajno smanjen, od 0.33-1.64 log i 1.13-2.04 log na trupovima goveda i svinja, u usporedbi s periodom pre-HACCP-a. Učestalost EC u uzorcima i nivou EC u EC-pozitivnim uzorcima su bili također umanjeni tijekom post-HACCP perioda u usporedbi s pre-HACCP periodom, ali redukcije nisu bile statistički značajne uslijed velike proporcije EC-negativnih uzoraka i vrlo niskih brojeva kod EC-pozitivnih uzoraka. Prisutnost *Salmonella* spp. nije bila utvrđena ni u jednom uzorku sa trupova goveda ili svinja, bez obzira jesu li su uzorci uzeti tijekom pre- ili post-HACCP perioda. Sveukupno, efekt unapređenja procesne higijene kod primjene ne-interventnog HACCP sistema je dokazan kroz redukciju TVC na trupovima, ali ne bi mogao biti verificiran u ovoj studiji kroz slične redukcije EC i/ili salmonela, zbog njihovih niskih nivoa i/ili odsustva.

**Ključne reči:** ne-interventni HACCP, mikrobiologija trupova, procesna higijena

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## Effetto dell'applicazione dell'HACCP non-interventivo sugli indicatori d'igiene di processo degli addomi bovini e suini

### Sommario:

Da ciascuno di 720 addomi trattati (360 bovini e 360 suini), sono stati presi i campioni da quattro posti nell'addome (in totale 2 880 campioni), in una commerciale macelleria di buoi e maiali, da due linee separate. I campioni sono stati presi dagli addomi prima di applicazione del sistema HACCP (pre-HACCP; 960 campioni), dopo l'applicazione del sistema HACCP (post-HACCP; 1 920 campioni), con lo scopo di determinare il numero totale di batteri (TVC), il numero di *Enterobacteriaceae* (EC), e la prevalenza di *Salmonella* spp. Durante il periodo pre-HACCP, il numero medio totale di batteri, da quattro esminati posti dall'addome, negli addomi bovini ha dimostrato la differenza entro 3.03 e 4.19 log<sub>10</sub> cfu/cm<sup>2</sup>, e negli addomi suini entro 3.73 e 3.99 log<sub>10</sub> cfu/cm<sup>2</sup>. Durante il periodo post-HACCP, il numero totale da tutti i punti di addome testati era notevolmente ridotto, da 0.33-1.64 log e da 1.13-2.04 log negli addomi bovini e suini, e rispettivamente comparando il periodo pre-HACCP. La frequenza EC nei campioni e i livelli EC nei campioni EC-positivi sono anche stati diminuiti durante il periodo post-HACCP, paragonando con il periodo pre-HACCP, ma le riduzioni non erano statisticamente notevoli grazie alla grande proporzione di campioni EC-negativi e i numeri molto bassi dai campioni EC-positivi. La presenza di *Salmonella* spp. non è stata determinata in nessuno dei campioni presi dagli addomi bovini o suini, nonostante il fatto che i campioni erano presi durante i periodi pre- o post-HACCP. Si può concludere che l'effetto di miglioramento dell'igiene del processo nell'applicazione del sistema non-interventivo è stato confermato tramite la riduzione TVC di addomi, ma non potrebbe essere verificato in questo studio tramite le riduzioni simili EC o/e *Salmonella*, per i loro livelli bassi, oppure la loro assenza.

**Parole chiave:** non-interventivo HACCP, microbiologia di addome, igiene del processo

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## Anwendungseffekt von "nichtinterventen" HACCP-Systemen auf Indikatoren der Prozesshygiene auf Rind- und Schweinekadaver

### Zusammenfassung

Von jedem der 720 behandelten Tierkadaver (360 Rind- und 360 Schweinekadaver) wurden Muster auf vier Stellen (insgesamt 2,880 Muster) in einem kommerziellen Schlachtwerk, von zwei verschiedenen Schlachtlinien, genommen. Die Muster wurden vor der Anwendung des HACCP Systems (vor-HACCP; 960 Muster) und nach der Anwendung des HACCP Systems (post-HACCP; 1,920 Muster) genommen. Es wurde die Gesamtzahl der Bakterien (TVC), die Zahl der *Enterobacteriaceae* (EC) und Prävalenz *Salmonella* spp. bestimmt. Im Laufe der vor-HACCP Periode unterschied sich die mittlere Gesamtzahl auf vier testierten Stellen am Kadaver, u. zw. auf Rindkadaver zwischen 3.03 und 4.19 log<sub>10</sub> cfu/cm<sup>2</sup> und auf Schweinekadaver zwischen 3.73 und 3.99 log<sub>10</sub> cfu/cm<sup>2</sup>. Im Laufe der post-HACCP Periode war die Gesamtzahl der Bakterien auf allen testierten Stellen der Kadaver bedeutend reduziert, von 0.33-1.64 log und 1.13-2.04 log auf Rind- und Schweinekadaver, respektiv, im Vergleich mit der vor-HACCP Periode. Die Häufigkeit von EC in den Mustern und die Niveaus von EC in EC-positiven Mustern war auch vermindert im Laufe der post-HACCP Periode im Vergleich mit der vor-HACCP Periode, aber die Reduktionen waren statistisch nicht bedeutend infolge von der großen Proportion von EC-negativen Mustern und sehr niedrigen Zahlen bei den EC-positiven Mustern. Die Anwesenheit von *Salmonella* spp. war auf keinem Muster der Rind- und Schweinekadaver vorgefunden, ohne Rücksicht darauf ob die Muster vor-HACCP Periode, während oder nach-HACCP Periode genommen wurden. Insgesamt wurde der Effekt der Förderung der Prozesshygiene bei der Anwendung des nicht-interventiven HACCP Systems durch die Reduktion von TVC auf Kadaver bestimmt, jedoch kann er in dieser Studie durch ähnliche Reduktionen von EC und/oder *Salmonella* wegen ihrer niedrigen Niveaus und/oder Nichanwesenheit nicht verifiziert werden.

**Schlüsselwörter:** nicht-interventives HACCP, Mikrobiologie der Kadaver, Prozesshygiene