

Pigs and cattle slaughter process hygiene in a large-scale and a small-scale abattoir: A report from one county in Serbia

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Abstract: Microbiological data on hygiene indicators are important for assessment of hygiene levels in abattoirs and control of carcass contamination. Risk categorization of slaughterhouses should be based on a process hygiene output through the use of indicator organisms – Total Viable Counts (TVC), Enterobacteriaceae counts (EC) and *Salmonella* spp. – monitored on carcasses. The level of TVC indicates the overall hygiene in abattoirs (equipment, tools, workers), while the presence of EC on carcasses can indicate fecal contamination which can occur during slaughter/dressing. Detection of *Salmonella* spp. indicates the presence of pathogens, with potential origin from the farm. The aim of this study was to assess the slaughter process hygiene in abattoirs of different sizes and throughputs, a large- and a small-scale abattoir; and to analyze differences in process hygiene levels based on implementation of their self-control plans, throughout a period of five years for cattle and six years for pigs. In the large-scale abattoir, TVC levels were slightly higher on cattle carcasses than on pig carcasses, but were always within the regulatory satisfactory or acceptable ranges. In the small-scale abattoir, low counts of EC were observed on cattle and pig carcasses, with slightly higher levels on cattle carcasses, but the counts were always within the regulatory satisfactory range. Higher TVC levels on both cattle and pig carcasses were observed in the large-scale abattoir versus the small-scale abattoir, but both abattoirs still showed process hygiene levels within the regulatory satisfactory or acceptable range. *Salmonella* was recovered only from two pig carcasses in the large-scale abattoir. The study revealed that TVC levels differed more on pig carcasses, while EC levels differed more on cattle carcasses. Process hygiene levels in both abattoirs were always in the allowed regulatory range and also were similar to the hygiene levels in other, developed European countries.

Keywords: abattoir, process hygiene, Total Viable Count, Enterobacteriaceae, *Salmonella*.

Introduction

An abattoir is a place where animals are humanely killed, under the supervision of authorized/official veterinarian, in order to provide meat for human consumption. During slaughter, many processing steps can contribute to cross-contamination of carcasses with the microorganisms originating from animal hide/skin, utensils and equipment, food-contact surfaces, workers and, most importantly, from the gastro-intestinal tract (*Veterinary Directorate*, 2007). Evisceration is the phase that contributes most to the finding of bacteria on the surface of the carcasses, especially because after skinning, at the slaughter line there is no longer any primary treatment phase that could reduce the number of bacteria (*Raseta et al.*, 2015).

The implementation of Good Manufacturing Practice (GMP), Hazard Analysis and Critical Control Points (HACCP) and various interventions

such as physical interventions (hygienic de-hiding, scalding/singeing/polishing, evisceration) and chemical interventions (carcass decontamination) (*Antic*, 2010) by slaughter and meat processing facilities play a large role in enhancing the safety of meat products (*Bohaychuk et al.*, 2011). Such interventions can lead to significant decrease of microbial numbers on carcasses. However, the physical methods cannot be substituted and/or replaced simply by chemical interventions, but rather must be supplemented with multiple decontamination procedures which have additional biocidal effect and therefore increase the level of microbial reductions. Additionally, contamination from the environment can also be significant during primary processing at abattoirs (*Australian Meat Processor Corporation*, 2013).

From the standpoint of food spoilage and food-borne disease, enteric bacteria are of great concern because they are frequently encountered in red meat production. Although various foods can

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serve as vehicles of foodborne illness, meat and meat products remain important sources of human infections with a variety of foodborne pathogens, i.e. *Salmonella* spp., *Campylobacter jejuni/coli*, *Yersinia enterocolitica*, human pathogenic Verotoxigenic *Escherichia coli*, and *Listeria monocytogenes* (Nørrung et al., 2009). Foodborne disease provoked due to the presence of pathogenic bacteria in the food usually manifests itself in episodes of gastro-intestinal disease (diarrhea, vomiting, etc.) (Nørrung et al., 2009).

The intestines of animals contain large numbers of microorganisms, e.g. *E. coli* levels are usually greater than 10^5 cfu g⁻¹, and amongst these microorganisms can be found foodborne pathogens such as *E. coli* O157, *Salmonella* and *Campylobacter* (Australian Meat Processor Corporation, 2013). The important meat hygiene indicators are *Enterobacteriaceae* (EC) and Total Viable Counts (TVC). The deep muscle tissues of healthy, slaughtered livestock contain few, if any, microorganisms (Veterinary Directorate, 2007). However, their exterior surfaces (hide, hair, skin) are naturally contaminated with a variety of microorganisms, as well as their gastro-intestinal tracts. The most common order of event anticipating foodborne diseases involves the existence of a primary source, e.g. healthy animals intended for meat production, which can intermittently fecally shed the pathogens that can be further spread in the process of primary production (on-farm), processing (in abattoir and meat processing), as well as handling by consumers (Nørrung and Buncic, 2008).

Regulation (EC) No. 2073/2005 and the Regulation on the general and specific food hygiene requirements at any stage of production, processing and trade (Serbia, 2010) require the obligatory control of TVC and EC, which are defined as hygiene indicators in slaughter processes. Martelli et al. (2017) stated that TVC are defined as indicators of the overall slaughter hygiene (equipment, tools, workers, environment), while EC are recognized as indicators of fecal contamination on carcasses and in abattoirs. *Salmonella* is the second most commonly reported zoonotic gastro-intestinal pathogen in the European Union (EFSA, 2016), and a significant proportion of the cases is linked to the consumption of contaminated pork (Martelli et al., 2017). *Salmonella* is regularly found in the intestines of humans and animals, and by fecal shedding it can also reach the environment. Many *Salmonella* serovars which are closely related to foodborne outbreaks, e.g. *S. Typhimurium*, *S. Enteritidis*, *S. Newport*, and *S. Heidelberg*, have reservoirs in farm animals (Andino, 2015). The most common primary reservoirs for *S. Typhimurium* are

pigs, cattle and poultry (Nørrung et al., 2009). Pork is especially important because pigs are one of the main meat-producing species that can asymptotically carry *Salmonella*, periodically shedding the bacteria through feces (Martelli et al., 2017).

It has been estimated that approximately 1% of *Salmonella* infections in humans are caused by the consumption of contaminated pork or processed foods derived from pork in the United States (Guo, 2011), and 0.02 % in the EU (EFSA, 2016). On the other hand, the average percentage of all positive cattle carcasses contaminated with *Salmonella* in the EU along the meat chain (pre-harvest/on-farm, harvest/slaughter and post-harvest/meat-processing), during 2008 and 2009 was 0.2% (EFSA, 2013). The most commonly isolated *Salmonella* serovars originating from cattle carcasses were *S. Typhimurium* and *S. Dublin*. According to EFSA report from 2015, the overall herd prevalence was 12.4% and 2.1%, for pigs and cattle, respectively, while *Salmonella* was detected in 1.7% and 0.2% of pork and cattle meat samples, respectively (EFSA, 2016).

In addition, the main reservoir of *E. coli* O157 is cattle, which can shed the pathogen fecally, and therefore, this alimentary pathogen can subsequently contaminate the foods originating from cattle, most commonly, meat. Direct fecal contamination of carcass with pathogens originating from penetrated intestines during evisceration (leakage from guts onto the meat) is relatively rare in modern slaughterhouses, while microbiological contamination from hides (direct contact, knife, equipment, air) is a crucial and relatively common event (Antic et al., 2010; Koohmaraie et al., 2005). In Serbia, Nastasijevic et al. (2008) found that the prevalence of *E. coli* on the skin of slaughtered cattle was 28.2%, while Blagojevic et al. (2011) found that the prevalence was 52 to 64%. Under conditions of simulated direct skin-meat contact, this transfer amounts to 0.5% of TVC and 2% of EC from the numbers on cattle skins (Antic et al., 2010). However, despite this low transfer rate, the high level of bacterial contamination of the skin, as well as the regularity of bacterial transfer to the body during skin removal, indicate that the risk of bacterial contamination of the carcass from the skin is very significant (Nastasijevic et al., 2016).

According to the 1989 regulation on the conditions that must be fulfilled by abattoirs and meat processing establishments (SFRJ, 1989) and until 2011, abattoirs in Serbia were divided by structure, technical equipment, capacity, work and veterinary inspection organization into: (i) large-scale abattoirs (industrial abattoirs), (ii) small-scale abattoirs, and (iii) community abattoirs. However, meat

produced in small abattoirs can have a significant influence on consumers taking into consideration the level of their exposure to the meat delivered by these abattoirs, based on the market share. One of the best examples is in the UK, where 51% of abattoirs are small-scale, accounting for 22.7% of the meat market share (UK, 2008).

Changes to the Serbian Rulebook on Veterinary-Sanitary Requirements, and general and special conditions of hygiene of food of animal origin (Serbia, 2011) and its harmonization with EU law (EC, 2004) on the hygiene of foodstuffs of animal origin, did not recognize differences between large- and small-scale abattoirs. In fact, the terms *large-scale abattoirs* and *small-scale abattoirs* has been used to describe production volume, the number of employees, the craft of financial assets and the annual profit. For example, small-scale UK abattoirs have a maximum of 50 employees, while large establishments have a minimum of 250 employees (UK, 2008). No matter whether the abattoir is small-scale or large-scale, it must satisfy specific conditions related to the construction methods, technical/technological equipment, veterinary/sanitary conditions, working methods, hygiene level and workers' training. The aim of this research was: a) to determine if there are any differences regarding process hygiene level at slaughter between selected large- and small-scale abattoirs in one Serbian county, and b) to assess to which extent those abattoirs achieved satisfactory level of the slaughter process hygiene.

Materials and methods

The study encompassed one small-scale and one large-scale (industrial) abattoir in one county in Serbia, regularly inspected by the competent authority (veterinary inspection). Abattoirs that were the object of this research had different production capacities. The industrial abattoir had a daily production capacity of 700 pig carcasses and 70 cattle carcasses. Daily capacity of the small-scale abattoir was 90 pig carcasses and 30 cattle carcasses. Altogether, 1180 wet-dry swabs were collected in the large- and small-scale abattoirs during a five year period (2012–2016) for cattle carcasses and during a six year period (2011–2016) for pig carcasses. Therefore, swab (sample) collection took place throughout 2011 and until 2016. Samples were taken in accordance with the local regulation on the general and specific food hygiene requirements at any stage of production, processing and trade (Serbia, 2010). A random sampling strategy was followed, which means that swabs were regularly collected

once per month. The sampling was based on standard Serbian-ISO harmonized methods (ISO, 2009). The sampling was also in line with the self-control plans developed by HACCP teams in both abattoirs and was regularly approved by the veterinary inspector in charge. The number of samples by year was not the same for large- and small-scale abattoirs, due to observed differences in their self-control plans. Therefore, the sampling frequency varied for large- and small-scale abattoirs depending on the year during the period 2011–2016. The sampling of cattle and pig carcasses was conducted by an authorized person in both abattoirs and testing was performed by an external laboratory accredited in accordance with ISO 17025 (ISO, 2005) for TVC, EC and *Salmonella* detection and enumeration. TVC and EC detection was performed according to ISO 4833 (ISO, 2003) and ISO 21528–2 (ISO, 2004), respectively. The interpretation of results was carried out according to EU Regulation 2073/2005 (EC, 2005). Detection of *Salmonella* spp. was carried out according to ISO 6579 (ISO, 2002), and results were recorded as *Salmonella* presence or absence (EC, 2005).

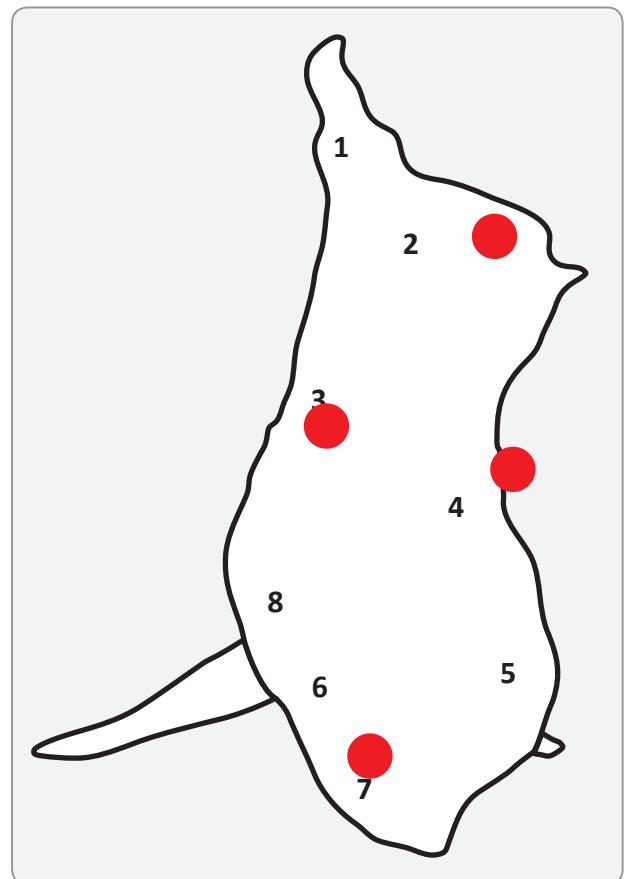


Figure 1. Sampling sites on pig carcass

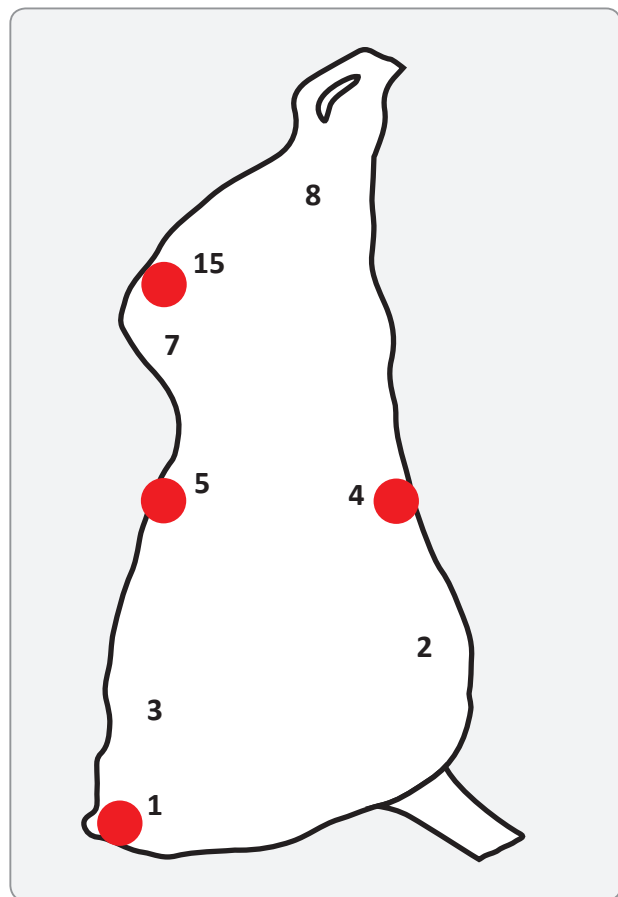


Figure 2. Sampling sites on cattle carcass

Swabs were collected in each abattoir at regular time intervals during this study. During each sampling session, swabs were taken from five cattle and/or five pig carcasses randomly selected at the end of slaughter line, after the final wash but before chilling. The swabs were taken at four sites on each carcass, e.g. pig carcasses (rump, belly, back and jaw; Figure 1) and cattle carcass (rump, belly, thorax

and neck; Figure 2) following the recommendation of the harmonized national standard (ISO, 2009; it has to be taken into consideration that this version of the standard was used during the time when this study was conducted, from 2011–2016).

Sterile, pre-moistened sponges were used to swab four adjacent areas (Figure 1) covering a total area of 400 cm² (100 cm² per each area) on one half of each chosen carcass. *Salmonella* was recovered from sponges used to swab the corresponding half of each carcass, from a 400 cm² area. In total, 1180 carcasses were examined from 2011–2016. Altogether, 640 carcasses (cattle n=270, pig n=370) were swabbed in the industrial, large-scale abattoir, while 540 carcasses (n=cattle 340, pig n=200) were swabbed in the small-scale abattoir.

The obtained microbial results/data were analyzed using Microsoft Office Excel 2007. Firstly, the average logarithm value of TVC and EC for each carcass was calculated (based on previously estimated log values for each of four corresponding sites on each carcass), and then the average daily logarithm was defined. The average daily logarithm of *Salmonella spp.* was not calculated, taking into account the regulatory requirement defining only the absence or presence of *Salmonella spp.*

Results

Overall, in the period from 2012 to 2016 for cattle carcasses and 2011 to 2016 for pig carcasses, TVC counts ranged from 1.17–2.33 log cfu cm⁻¹ and from 1.34–3.10 log cfu cm⁻¹, for cattle and pig carcasses, respectively (Tables 1 and 2). The EC levels varied from 0.01–0.37 log cfu cm⁻¹ and 0.11–0.82 log cfu cm⁻¹, for cattle and pig carcasses, respectively (Tables 1 and 2).

Table 1. The level of cattle carcass contamination in a large-scale and a small-scale abattoir, 2012–2016 (n=610)

	2012		2013		2014		2015		2016	
	A	B	A	B	A	B	A	B	A	B
n	85	55	50	75	35	85	55	50	75	35
%	31.48	16.18	18.52	22.06	12.96	31.48	16.18	18.52	22.06	12.96
TVC – log cfu cm ⁻¹ (\bar{x} ±SD)	0.60±0.26	0.24±0.15	1.12±0.10	0.82±0.19	1.63±0.11	1.43±0.17	2.16±0.21	1.99±0.14	2.85±0.25	2.57±0.23
EC – log cfu cm ⁻¹ (\bar{x} ±SD)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.04	0.00±0.00	0.17±0.04	0.11±0.12	0.97±0.66	0.84±0.38
<i>Salmonella spp.</i>	n	0	0	0	0	0	0	0	0	0
	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Legend: A – large-scale abattoir; B – small-scale abattoir, n – number of carcasses

Table 2. The level of pig carcass contamination in a large-scale and a small-scale abattoir, 2011–2016 (n=570)

	2011		2012		2013		2014		2015		2016	
	A	B	A	B	A	B	A	B	A	B	A	B
n	35	5	55	20	50	35	90	50	80	35	60	55
%	9.46	2.50	14.86	10.00	13.51	17.50	24.32	25.00	21.62	17.50	16.22	27.50
TVC – log cfu cm ⁻¹ (\bar{x} ±SD)	0.39±0.18	0.00±0.00	1.15±0.12	0.47±0.33	1.44±0.07	1.01±0.18	1.94±0.27	1.60±0.25	2.63±0.17	2.26±0.14	3.40±0.28	3.06±0.39
EC – log cfu cm ⁻¹ (\bar{x} ±SD)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.37±0.14	0.89±0.71	1.30±0.55	0.06±0.10
<i>Salmonella</i> spp.	n	0	0	0	0	0	0	0	2	0	0	0
	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00

Legend: A – large-scale abattoir; B – small-scale abattoir, n – number of carcasses

Total Viable Counts

In the large-scale abattoir, TVC were isolated from all cattle carcasses (n=300; 100%) and from all pig carcasses (n=370, 100%). Mean TVC values for cattle carcasses were within the satisfactory range (<2.8 log cfu cm⁻¹) in 75% (n=225) of tested cattle carcasses, while in 25% (n=75) of tested carcasses, TVC levels were within the acceptable range, between 2.8–4.0 log cfu cm⁻¹. The established TVC levels on pig carcasses were within the satisfactory range (<3.3 log cfu cm⁻¹) in 74.33% (n=275) of tested carcasses, while in 25.67% (n=95) of tested pig carcasses, the TVC levels were within the acceptable range, between 3.3 and 4.3 log cfu cm⁻¹. In the small abattoir, TVC were isolated from all cattle carcasses (n=300; 100%), with levels within the satisfactory range. Further, TVC were isolated from all tested pig carcasses (n=200, 100%), but again, all of them were within the satisfactory range. Apparently higher TVC levels were observed in the large-scale abattoir versus the small-scale abattoir, but both abattoirs still showed process hygiene levels within the satisfactory range (Table 3).

Enterobacteriaceae Counts

In the large-scale abattoir, EC were isolated from 165 (55%) cattle carcasses, while on 135 carcasses, the EC levels were below the detection limit; EC values were always within the satisfactory range, below 1.2 log cfu cm⁻¹. On 230 (62.16%) of pig carcasses, the EC levels were below the detection limit, while on 140 (37.84%) of pig carcasses, the detected EC levels were within the satisfactory range (<1.3 log cfu cm⁻¹). In the small abattoir, low counts of EC were also determined. EC were confirmed on 85 (28.33%) of the cattle carcasses and 90 (45%) of the pig carcasses. In both cases, cattle and pig carcasses, the observed EC levels were always within the satisfactory range as defined by the legislation (EC, 2005; Serbia, 2010).

Salmonella species

The presence of *Salmonella* spp. was detected only on two pig carcasses originating from the large-scale, industrial abattoir.

Table 3. Summary view of cattle and pig carcass contamination in a large-scale and a small-scale abattoir, 2011–2016 (n=1180).

	TVC – log cfu cm ⁻¹ (\bar{x} ±SD)		EC – log cfu cm ⁻¹ (\bar{x} ±SD)		<i>Salmonella</i> spp. detected	
	Cattle	Pig	Cattle	Pig	Cattle	Pig
A	(n=270)	(n=370)	(n=270)	(n=370)	(n=270)	(n=370)
	1.53±0.98	1.99±1.01	0.20±0.49	0.30±0.62	0	2*
B	(n=340)	(n=200)	(n=340)	(n=200)	(n=340)	(n=200)
	1.40±0.97	1.86±1.10	0.17±0.47	0.26±0.61	0	0

Legend: A – large scale abattoir; B – small scale abattoir; n – number of carcasses *during 2015, *Salmonella* spp. was detected on two pig carcasses.

Discussion

TVC is the general indicator for hygienic operations indicating the overall hygiene in abattoirs (equipment, tools, workers), while EC counts indicate fecal contamination of carcasses. The TVC/EC levels detected on the carcasses do not serve *per se* for decision-making on carcass/meat acceptance or rejection, but rather serve as general indicators of slaughter hygiene (Delhalle et al., 2008). Low levels of TVC were detected at all times, either within the satisfactory or acceptable range. In both abattoirs, TVC on cattle carcasses were always within the regulatory requirements. Some other studies reflecting TVC levels on cattle carcasses showed different variations, such as a study carried out in Ethiopia where TVC levels were 5.21 log cfu cm⁻¹ (Gebeyehu et al., 2013), in Algeria, 4.48 log cfu cm⁻¹ (Nouichi and Hamdi, 2009), in Switzerland, between 2.1–3.1 log cfu cm⁻¹ (Zweifel et al., 2005) and in Australia, 2.42 log cfu cm⁻¹ (Phillips et al., 2001).

In both abattoirs, the EC levels were at all times within the satisfactory range for cattle and pig carcasses, respectively. A similar result was reported in another study conducted in Serbia, where the slaughter process hygiene was assessed in two large- and two small-scale abattoirs, accounting for 58.5% of Serbia's national production of beef/pork meat (Nastasijevic et al., 2016). Our low *Salmonella* prevalence was also in accordance with an earlier study carried out in Serbian abattoirs (Blagojevic et al., 2011). In the current study, *Salmonella* was absent from cattle carcasses, while it was detected on two pig carcasses in the large-scale abattoir. It is worth noting the current legislation (EC, 2005; Serbia, 2010), by which the presence of *Salmonella* on 2 of 50 cattle carcasses and on 5 of 50 pig carcasses is acceptable.

Also, based on available results from developed countries such as the USA and Ireland, as well as other countries such as Algeria and Turkey, where average contamination of bovine carcasses was 1.5% (Sofos, 2005), 7.6% (Keogh et al., 2001), 10% (Nouichi and Hamdi, 2009) and 10% (Akkaya et al., 2008), respectively, it can be concluded that slaughter hygiene in the chosen Serbian slaughterhouses was similar or better than that in some other developed countries.

It is worth noting that industrial-type, large-scale production meat establishments mostly have more developed risk-based meat safety management systems (HACCP-based) and more intensive cooperation with professional/scientific institutions and laboratories compared with small-scale establishments. This fact can be sometimes related

to the higher level of slaughter process hygiene in large-scale meat establishments versus small-scale establishments. On the other hand, it should be kept in mind that small abattoirs with a smaller slaughter throughput have more opportunities to thoroughly conduct cleaning and disinfection protocols and to devote more attention to monitoring critical control points, which subsequently contributes to the higher level of hygiene.

Conclusion

Microbiological data on hygiene indicators are important for assessment of hygiene levels in abattoirs and control of carcass contamination. Risk categorization of abattoirs should be based on a process hygiene output through the use of indicator organisms, TVC and EC, monitored on carcasses. The indicators monitored on carcasses (TVC, EC) indicate whether the process hygiene functions acceptably, but they do not indicate control of the hazards *per se*. The presence of TVC indicate the overall hygiene in abattoirs (equipment, tools, workers), while the presence of EC on carcasses can indicate fecal contamination which can occur during slaughter/dressing. In addition, *Salmonella* spp. can serve as an indicator of the presence of pathogens on carcasses/meat. In this study, we compared the process hygiene levels between two selected abattoirs, a large-scale and a small-scale abattoir, over a period of five years for cattle carcasses and six years for pig carcasses. In total, 1180 cattle or pig carcasses were examined. The results revealed that in the large-scale abattoir, TVC were isolated from all cattle and pig carcasses. Mean TVC values for cattle carcasses were within the satisfactory range in 75% (n=225) of tested carcasses, while for 25% (n=75) of carcasses, TVC levels were within the acceptable range. TVC levels on pig carcasses were within the satisfactory range in 74.33% (n=275) of tested carcasses, while for 25.67% (n=95) of pig carcasses, TVC levels were within the acceptable range. In the small abattoir, TVC were also isolated from all cattle/pig carcasses, with levels always within the satisfactory range. Apparently higher TVC levels were observed in the large-scale abattoir versus the small abattoir, but both abattoirs still showed process hygiene levels were within the regulatory satisfactory range. In the large-scale abattoir, EC were isolated from 55% (n=165) of cattle carcasses and were always within the satisfactory range. On 62.16% (n=230) of tested pig carcasses, the EC levels were below the detection limit, while on 37.84% (n=140) of pig carcasses,

the detected EC levels were within the satisfactory range. In the small abattoir, low EC levels were observed on cattle/pig carcasses and were always within the regulatory satisfactory range.

Salmonella spp. prevalence was low, as this pathogen was not detected on cattle carcasses over the five year period, while only two positive findings were observed among the pig carcasses over six years.

Overall, the observed process hygiene levels in both abattoirs did not differ significantly, and were

rather similar to process hygiene levels in other, developed EU countries. Development and vigorous implementation of self-control plans intended for monitoring hygiene indicators (TVC, EC) on cattle or pig carcasses can lead to achievement of satisfactory levels of slaughter hygiene, no matter the size and throughput of the abattoir. Further research should be carried out to establish an evidence-based interface between slaughter process hygiene, risk categorization of abattoirs and the frequency of inspection visits.

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