

A hygiene report regarding slaughter process of pig and cattle carcasses for 2017 in Serbia

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Abstract: For the successful validation and verification of the HACCP system, a food business operator is obliged, among other duties, to have continuous microbiological data of carcasses which are followed by a certain dynamic that the subject himself prescribed. To obtain these data, it is necessary to perform systematic monitoring of indicator microorganisms. The most important meat hygiene indicators are Total Viable Counts (TVC) and Enterobacteriaceae (EC). TVC are defined as indicators of overall slaughter hygiene (equipment, environment, tools, workers), while EC are indicators of faecal contamination on carcasses. The aim of this study was to determine the microbial contamination of cattle and pig carcasses, level of hygiene of the slaughter process as well as whether variations hygiene levels were related to seasonality in Serbia during 2017. The year was divided into four quarters of three months each, while the microbiological results were classified into three levels of hygiene status (unsatisfactory, satisfactory or acceptable). The highest percentage of the results surveyed during the entire study was at a satisfactory hygiene level. Furthermore, we found there were differences in results between the quarters, which could be associated with seasonality. The best microbiological results, and so the best hygiene of carcasses, was recorded in the period April, May, June, while the worst microbiological results were observed in the period of July, August and September.

Keywords: slaughterhouse, process hygiene, Total Viable Count, Enterobacteriaceae, seasonality.

Introduction

Meat consumption is increasing worldwide due to rapid population growth and urbanization. This has resulted in increased concerns and challenges when it comes to meat safety and hygiene. Therefore, the meat safety regulations should be implemented from farm, slaughterhouse, processing, storage, distribution, retail outlets up until the products reach the consumers (*Sofos and Geornaras*, 2010). During the food production process, each step of production is controlled and has a set of specific rules. The process of hazard analysis and critical control point (HACCP) is a systematic, science-based approach process control, designed to prevent, reduce or eliminate identified hazards in food products (*Kukay et al.* 1996). Therefore, a food business operator (FBO) is obligated to achieve compliance with these regulations. FBOs must apply compulsory self-checking programs following the HACCP approach.

The slaughterhouse is an indispensable part of the meat processing chain, being the place where animals are humanely killed to produce meat and meat

products, and the entire process is under the supervision of an official veterinarian. Codex Alimentarius guidelines deal with hygiene of animals presented for slaughter: "the degree of contamination of the external surfaces of the animal is likely to compromise hygienic slaughter and dressing, and suitable interventions such as washing, or shearing are not available" (*Codex Alimentarius*, 2005). Regulation EU 853/2004 lists requirements that FBOs operating slaughterhouses must comply with, and one of them is that animals must be clean. Strict maintenance of good abattoir hygiene practices is of central importance to ensure both public health protection and meat quality. Implementation of good manufacturing practices (GMP) as well as HACCP for slaughterhouses and meat processing facilities plays a major role in enhancing the safety of meat products (*Bohaychuk et al.*, 2011). Generally, it is accepted that the HACCP approach is the most effective way of reducing or eliminating contamination during food processing. For successful implementation of a HACCP system, it is necessary to have data on microbiological contamination, since the carcasses

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can be contaminated despite the absence of visible dirt (Tergney and Bolton, 2006). Scientific research has shown that with regard to long-term hygienic conditions in abattoirs, regular microbiological examination of the carcasses provides reliable conclusions (Zweifel and Stephan, 2003). This microbiological data can also be used for compliance with abattoir-specific performance criteria (Zweifel et al., 2005). In order to obtain these data, it is necessary to perform systemic monitoring of indicator microorganisms (Brown et al., 2000).

Regulation (EC) No. 2073/2005 and Serbian Regulation (Official Gazette of the Republic of Serbia, No. 72/10, 2011) require the obligatory control of hygiene indicators in slaughter processes. The most important meat hygiene indicators are Total Viable Counts (TVC) and *Enterobacteriaceae* (EC). TVC are defined as indicators of overall slaughter hygiene (equipment, environment, tools, workers), while EC are indicators of faecal contamination on carcasses (Martelli et al. 2017). Following the indicator microorganisms, allows indirect monitoring of the presence of pathogenic microorganisms such as *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni/coli*, *Escherichia coli* and *Yersinia enterocolitica* (Nørrung et al., 2009). These food-borne pathogens are still considered as important sources of human infections (Nørrung et al., 2009).

Analysis of data which were collected from slaughterhouses in Serbia in 2017 was conducted to understand the microbiological contamination of cattle and pigs carcasses, as well as establish the level of hygiene of the slaughter process for each animal. Further analysis of the obtained data should establish whether there are any variations in the hygiene results and if they exist, any relationship with seasonality.

Materials and methods

Abattoirs and carcass sampling

The study encompassed abattoirs with different production capacities in Serbia, and which were regularly subjected to veterinary inspection. A total of 554 wetdry swabs were collected during one year for cattle carcasses and 769 swabs were collected for pig carcasses. Following national regulations on the general and specific food hygiene requirements, we took samples in the production and processing phases. The number of samples by month was in line with the selfcontrol plans developed by HACCP teams in every abattoir. Samples were obtained by the wet-dry swab technique from four different sites

(4 × 100 cm²; cattle: neck, brisket, flank, and rump; pig: back, cheek, ham, and belly) of one half of each chosen carcass, as stipulated by SRPS ISO 17604 (ISO, 2016). Sampling was conducted by an authorized and trained laboratory technician and 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. Sample testing was performed by an ISO 17025 (ISO, 2005) accredited laboratory in accordance with respective ISO methods for TVC and EC enumeration.

TVC and Enterobacteriaceae enumeration

Each sample, consisting of four swabs, was added to 100 mL BPW (Oxoid, UK) and homogenized for 1 min in a stomacher (Laboratory Blender, Stomacher 400). TVC was obtained by inclusion of 1 mL of the suspension (M) and a series of 10-fold dilutions (10⁻¹, 10⁻², 10⁻³) in duplicate into Plate Count Agar plates ([PCA] Oxoid, UK). EC counts were obtained by spread plating 1 mL of the suspension (M) and a series of 10-fold dilutions (10⁻¹, 10⁻², 10⁻³) in duplicate onto Violet Red Bile Glucose Agar plates ([VRBGA] Oxoid, UK). Five characteristic colonies per plate were confirmed following SRPS ISO 21528-2:2009 (oxidase test and glucose fermentation test). The media was incubated as described above, according to SRPS EN ISO 4833-1:2014 and SRPS ISO 21528-2:2009. TVC and EC were calculated according to SRPS EN ISO 7218:2008/A1:2014. Bacterial recovery was expressed as log₁₀ colony-forming units (cfu)/cm² and calculated as described in SRPS ISO 18593:2010, using the following formula:

$$\text{cfu cm}^{-2} = N \times F / A \times d$$

N = the number of cfu in 1 mL of dilution liquid (or neutralizing liquid);

F = the amount, in millilitres, of dilution fluid (or neutralizing liquid) in the tube or homogenizer bag;

A = is the area swabbed, in square centimetres;

d = is the dilution corresponding

Analysis of results

The year was divided into four quarters of three months. The first quarter (Q1) was January, February and March, the second quarter (Q2) comprised April, May and June. The third (Q3) comprised July, August and September, while October, November and December made up the fourth quarter (Q4).

All carcasses were classified into the following hygiene categories: unsatisfactory, satisfactory

and acceptable, according to *Anonymous* (2017). For cattle carcasses, unsatisfactory results were those counts above 4.3 log cfu cm⁻² for TVC and above 1.8 log cfu cm⁻² for EC. Acceptable results were those counts below 4.3 log cfu cm⁻² for TVC and below 1.8 log cfu cm⁻² for EC. Satisfactory results were those equal to or below 2.8 log cfu cm⁻² for TVC and equal to or below 0.8 log cfu cm⁻² for EC. Pig carcasses were deemed unsatisfactory if they had more than 4.3 log cfu cm⁻² TVC and more than 2.3 log cfu cm⁻² for EC; acceptable pig carcasses had less than 4.3 log cfu cm⁻² for TVC and less than 2.3 log cfu cm⁻² for EC. Microbial levels on satisfactory pig carcasses were equal to or below 3.3 log cfu cm⁻² for TVC and equal to or below 1.3 log cfu cm⁻² for EC.

The obtained microbial results/data were analysed using Microsoft Office Excel 2016.

Results and Discussion

In Serbia in 2017, cattle carcass contamination levels ranged from 0.0 log cfu cm⁻² to 3.7 log cfu cm⁻² for TVC and from 0.0 log cfu cm⁻² to 2.4 log cfu cm⁻² for EC, while pig carcass contamination levels ranged from 0.0 log cfu cm⁻² to 3.88 log cfu cm⁻² for TVC and 0.0 log cfu cm⁻² to 2.7 log cfu cm⁻² for EC.

Cattle carcasses

TVC values for cattle carcasses

In Q1, TVC levels on cattle carcasses were within the satisfactory range (equal to or less than 2.8 log cfu cm⁻²) in 85.5% of tested cattle carcasses, while in 14.5 % of tested carcasses, TVC levels were within the acceptable range, between 2.8–4.3 log cfu cm⁻². In Q2, 92.86% of tested carcasses were

classified in the satisfactory hygiene group, and the remaining 7.14% were in the acceptable group. Altogether in Q3, 85.62% of carcasses belonged to the satisfactory group, while the rest (14.38%) were within the acceptable group. In Q4, we established that 82.71% of carcasses belonged in the satisfactory group, while the remaining 17.29% were classified in the acceptable group (Figure 1).

EC values for cattle carcasses

EC levels in Q1 were lower than 0.8 log cfu cm⁻² in 95.42% of tested carcasses, which therefore, belonged to the satisfactory group, while 3.05% had a level between 0.8 log cfu cm⁻² and 1.8 log cfu cm⁻², which put them in the acceptable group. Some cattle carcasses (1.53%) had an EC level higher than 1.8 log cfu cm⁻², and therefore, they belonged to the unsatisfactory group. In Q2, 97.14% of pig carcasses belonged to the satisfactory group and the remainder (2.86%) belonged to the acceptable group. In the third quarter, EC levels on cattle carcasses were within the satisfactory range in 86.93% of tested cattle carcasses, while in 7.84% of tested carcasses, EC levels were within the acceptable range and the remaining 5.23% of carcasses were within the unsatisfactory group. In Q4, 88.72% of tested carcasses belonged to satisfactory group, 7.52% of carcasses were within the acceptable range and 3.76% were classified as unacceptable (Figure 2).

Pig carcasses

TVC values for pig carcasses

The results of tested pig carcasses in Q1 showed that for TVC, 97% fell into the satisfactory group (equal to or less than 3.3 log cfu cm⁻²), while 3% belonged to the acceptable group (4.3–3.3 log cfu cm⁻²).

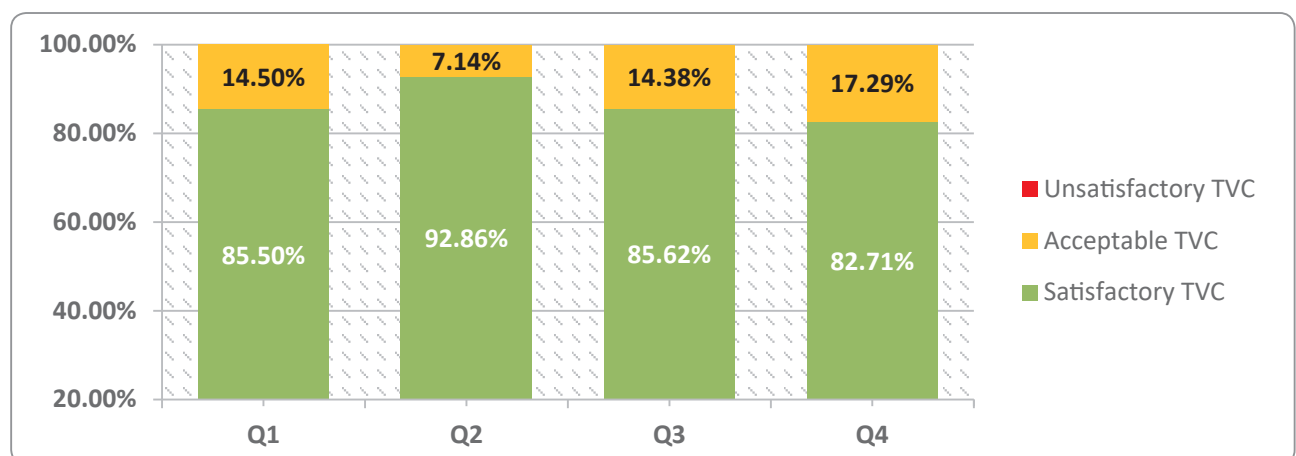


Figure 1. Trend analysis of Total Viable Count for cattle carcasses in 2017

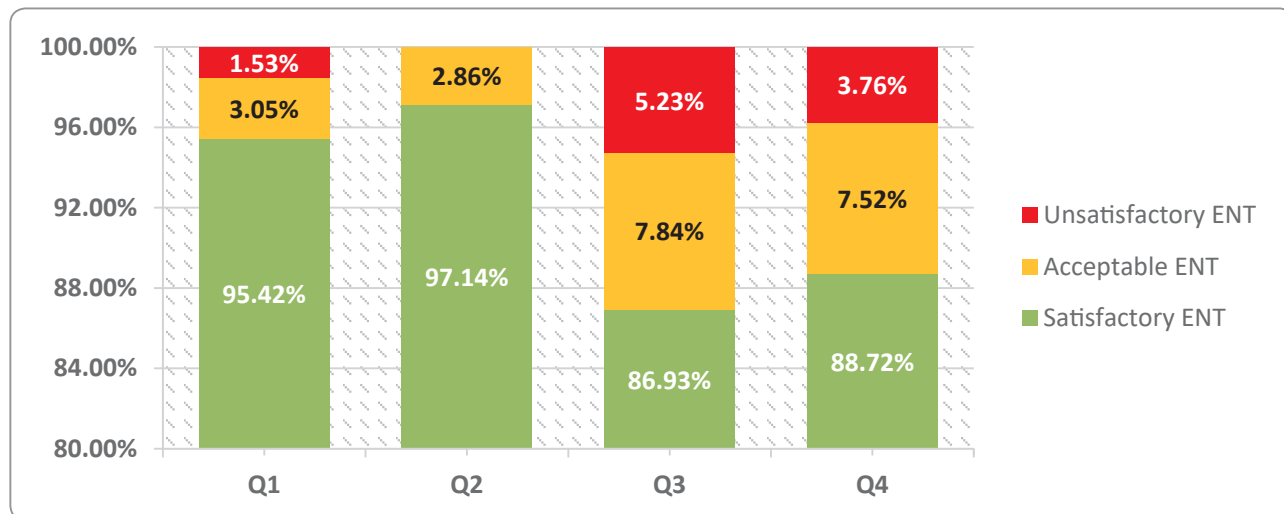


Figure 2. Trend analysis of *Enterobacteriaceae* for cattle carcasses in 2017

In Q2, 99% of tested carcasses were within the satisfactory group, and 1% were within the acceptable group. In Q3, 98% of tested carcasses belonged to the satisfactory group and 2% of pig carcasses belonged to the acceptable group. In Q4, 96% of pig carcasses belonged to the satisfactory group, while 4% of tested carcasses were within the acceptable group (Figure 3).

EC values for pig carcasses

EC values for pig carcasses in Q1 were such that 99% of tested pig carcasses belonged to the satisfactory group (equal to or less than 1.3 log cfu cm⁻²) and 1% of carcasses belonged to the acceptable group (1.3–2.3 log cfu cm⁻²). Similar results were observed in remaining quarters, where 99% of tested carcasses in Q2, 98% in Q3 and 96% in Q4 were within the

satisfactory group, while 1% of pig carcasses in Q2, 2% in Q3 and 4% in Q4 belonged in the acceptable group. In Q3, 1% of tested carcasses were classified in the unsatisfactory group (Figure 4).

TVC and EC are indicators for hygiene process in slaughterhouses (Delhalle et al., 2008). Following these two parameters indicates the overall hygiene (TVC) in the slaughterhouses, as well as faecal contamination (EC) of the carcasses. In 2017 (divided into four separate periods), we observed that over 82% of the studied cattle carcasses had satisfactory TVC levels ($\leq 2.8 \log \text{cfu cm}^{-2}$). However, the results were different for each quarter. Among the four annual quarters, Q2 had the highest percentage of carcasses with satisfactory hygiene status according to TVC levels. After that time, the percentage of carcasses with satisfactory hygiene status decreased

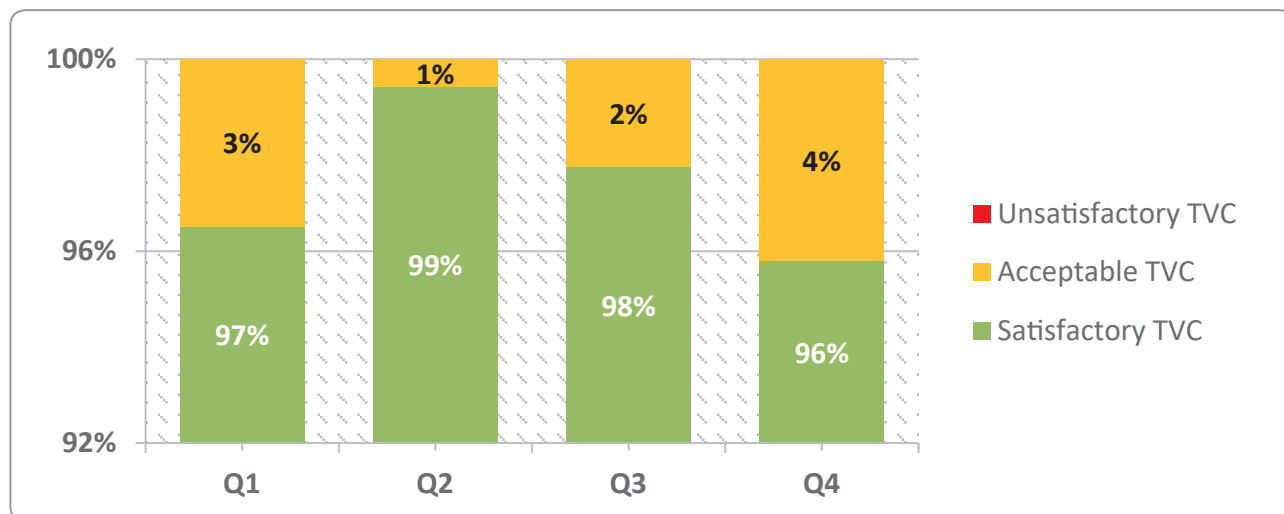


Figure 3. Trend analysis of Total Viable Count for pig carcasses in 2017

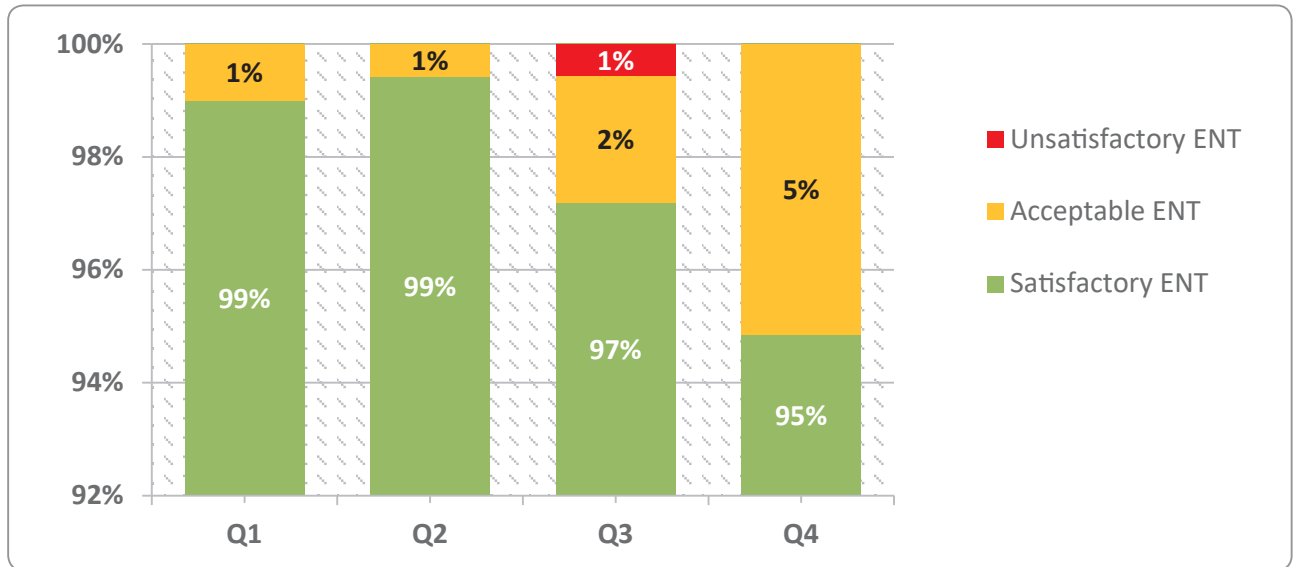


Figure 4. Trend analysis of *Enterobacteriaceae* for pig carcasses in 2017

during Q3 and Q4. Q4 had the highest percentage of carcasses with acceptable hygiene status according to TVC levels. Similar results were obtained for EC levels, where the highest percentage of carcasses with a satisfactory hygiene status was observed in Q2. Q1, Q3 and Q4 were periods when carcasses had unsatisfactory EC levels ($> 4.3 \log \text{cfu cm}^{-2}$), and this was especially true for Q3, when over 5% of carcasses had unsatisfactory EC levels. This difference between quarters can probably be related to the colder and warmer parts of the year. *Rony et al.* (2007) came to a similar conclusion in their research, in which they confirmed the differences in the microbial levels on carcasses between these two periods of the year. *Barco et al.* (2014) highlighted that the season appears to have a direct impact on EC counts on beef carcasses, associating low counts with the dry season and cold months. Every step from the farm, through transport, storage, general hygienic conditions in the slaughterhouse and activities before and during slaughter can have an impact, which can be seen by microbiological analysis. If we observe the farm, during the warmer months, animals use a higher amount of water; they also try to cool down in different ways. All of this results in a higher percentage of dirty animals, and therefore, a special focus on animals with dirt of faecal origin is required during the hotter, summer months. The transport of animals to slaughterhouses in the summer months should be given special attention, since any failure made during transport will have consequences on the microbiological results of the carcasses. The hide of dirty animals is the most important source of contamination for carcasses, in particular during skinning

operations. Bacteria including important foodborne pathogens can be transferred from hides onto carcasses via direct or indirect contact (*Brichta-Harhay et al.*, 2008). By the fact that the animals are dirtier, the possibility of contamination of the carcasses is greater. *Nastasijevic et al.* (2008) stated that in cattle carcass processing, one of the main concerns is related to observed soil and faecal contamination of carcasses and cross contamination during storage. The percentage of cattle carcasses which belonged to the unsatisfactory group in our study is probably because these carcasses had a greater potential for contamination with EC (*Zweifel et al.*, 2004). It should also be noted that slaughterhouses of different sizes were included in the current study.

Treatment of pig carcasses is different from cattle carcasses, because during pig processing, the carcasses pass through various characteristic and specific technological procedures. Due to this, microbiological contamination of pig carcasses is specific and one should expect different microbiological results and hygiene status compared to cattle carcasses. *Raseta et al.* (2015) obtained medium levels of $2.87 \log \text{cfu cm}^{-2}$ for TVC and $1.05 \log \text{cfu cm}^{-2}$ for EC levels on pig carcasses in a single slaughterhouse located in Banat, Serbia, for a period of one year. Out of all pig carcasses examined in our study, over 95% of them belonged to the satisfactory group. Observing TVC, the yearly period with the highest percentage of carcasses belonging to the satisfactory hygiene group was Q2, while the worst results occurred during Q4. Similarly poor results were obtained for EC in Q3, during which some carcasses were classified in the unsatisfactory group.

These results differ from the results of *Mrdovic et al.* (2017) who carried out research in one district in Serbia and found that TVC levels of pig carcasses were within the satisfactory range in 74.33% of tested carcasses and EC levels of carcasses were within satisfactory range in 100%, for a period of six years (2011–2016). These variations of results could reflect the improvement of hygienic conditions in slaughterhouses, which naturally, is an objective to strive for.

Conclusion

The hygienic conditions of slaughter are a focal point in the food chain. Despite the challenge of analysing results obtained from slaughterhouses of different sizes, the results obtained in this study indicate that the highest percentage of carcasses have satisfactory/acceptable levels of TVC and EC

contamination. These results showed that most of the examined slaughterhouses keep managing and maintaining high quality standards. However, the percentage of carcasses with higher levels of TVC and/or EC contamination, and so classified as unsatisfactory, shows there are still opportunities for improvement. Attention must be paid to every link in the food chain from the farm, through the transport, residence of the animals in lairage and on the slaughter processing line in order to achieve the best performance. Hygiene in a slaughterhouse must be at a high level, as must the education of workers who should have regular training in accordance with new scientific knowledge. A high degree of hygiene and quality should be maintained throughout the year. Special attention should be paid during the summer months, during which a higher percentage of carcasses classified as unsatisfactory from the point of view of microbial contamination was observed in this survey.

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