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Research Article

Evaluation of the microbiological quality of cattle carcasses in some slaughterhouses at Benin, West Africa

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ABSTRACT

Background: Slaughterhouse is one of the major critical points in meat hygiene with slaughtering being the stage of occurrence of most contamination risks.
Methods: This study aimed to assess the microbiological quality of cattle carcasses in the slaughterhouses of Cotonou/Porto-Novo. Samples were collected by excision from four parts on each carcass (neck, shoulder, flank and thigh). A total of eighty (80) samples from twenty (20) carcasses were analysed. Bacteriological analysis was achieved according to APC. ISO 4833: 2003; ISO 21528-2 and ISO 6579: 2002 norms, respectively for evaluate the aerobic plate count, enteric bacteria count, and qualitative detection of *Salmonella*.
Results: Results varied according to carcass parts, with no significant difference between the bacterial loads of these parts regarding the APC. However, a significant difference was observed between the load in enteric bacteria between the neck and the thigh. With respect to EC regulations (N° 2073/2005), the thigh is the most contaminated part with 100% unsatisfactory results for APC, as well as, enteric bacteria count together with high contamination by *Salmonella* (detected in 75% of thigh samples). Then following the shoulder, showing 100% unsatisfactory results for APC and enteric bacterial load with presence of *Salmonella* in 55% of samples.
Conclusions: Although being the most contaminated site, the inner part of the thigh had the lowest contamination level with respect to APC.

Keywords: Carcass, Slaughterhouse, Cattle, Surface contamination

INTRODUCTION

Contamination of foods from animal sources, mainly meat and meat products are responsible for 28% of cases of foodborne infections (TIAC).[1] Continuation of the problem is amply demonstrated in recent years by monitoring studies conducted on meat, for germs such as *Escherichia coli* O157: H7, *Salmonella spp.*, *Campylobacter spp.* and *Yersinia enterocolitica*.[2] In addition, slaughterhouse is one of the major critical points in meat hygiene with slaughtering being the stage of occurrence of most contamination risks.[3-8] According to Jouve, 80-90% of the micro-flora of meat reaching consumers come from contaminations occurred at the slaughterhouse.[9] Therefore, meat inspection services used by veterinarians at the slaughterhouse might not ensure the microbiological safety of meat.[10]

In Benin, not much research is focused on the microbiological quality of meat and the hygiene of slaughtering processes. Previous studies include those of Salifou et al. that showed instability of the hygienic quality of beef and therefore a lack of hygiene of the slaughtering process.[8,11,12] Current studies on the microbiological quality of inspected meats are thus needed in order to update these results and draw a final conclusion for appropriate safety actions. Moreover, studies in Benin focussing on the identification of carcass parts that are mostly exposed to contamination are very rare in the published literature.

The purpose of this study is to assess the microbiological quality of cattle carcasses in the slaughterhouses of Cotonou/Porto-Novo. In compliance with the ISO 17604 standard, Regulation (CE) N° 2073/2005 and technical information specified in service note DGAL/SDSSA/N2007-8275.[13-15]

METHODS

Sampling

Samples were taken randomly one day per week on five (5) semi-carcasses from five (5) different carcasses. From one sampling session to another, samples were collected from five (5) right semi-carcasses (5) and the left ones alternately. The sampled carcasses were randomly selected at the weighing station. Four (04) parts were taken: the neck (A), the thorax around the area near the shoulder (B), the outer part of the flank (C) and the inner part of the thigh (D) according to those defined by memo DGAL/SDSSA/N2007-8275 (Figure 1).[15]

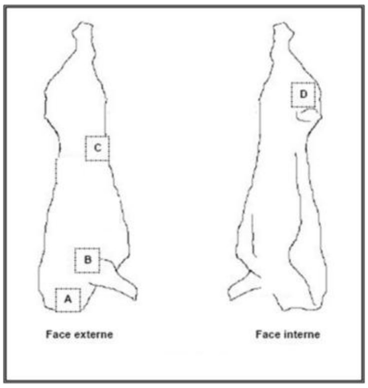

Figure 1: Illustration of the sampling sites on cattle carcass from service note DGAL/SDSSA/N2007-8275.[15] A: neck, B: the thorax in the shoulder portion (shoulder), C: the outer face of the side (Flank), D: the inner face of the thigh.

The sampling method adopted is a destructive method which consists of taking from each part of the carcass, 5 cm^2 muscle. For this purpose, a template cutting surface of 5 cm^2 was used. The template was placed on the sampling site. The outer edges of the template were excised with a sterilised scalpel. The piece of 5 cm^2 muscles was cut using a scalpel and a sterile forceps to get a thickness of 2 mm. The sample was then placed in a test tube which was then closed and placed in a cooler box whereby the temperature was maintained between 0 and 4°C. The cooler boxes were transported to the National Laboratory for Quality Control of Medicines and Medical Consumables of the Ministry of Health where samples were stored in a refrigerator (0-4°C) till culture. In total, eighty (80) samples from twenty (20) carcasses were collected.

Microbiological analysis

Bacteriological study was carried out to evaluate the aerobic plate count (APC, ISO 4833: 2003), enteric bacteria count (ISO 21528-2) and the qualitative detection of *Salmonella* (ISO 6579: 2002).[16-18] For the first two parameters, the results were expressed as CFU/centimetre square of surface (according to ISO 18593 standard, June 2004).[19] For *Salmonella*, results are reported according to presence or absence then presented as proportions.

Statistical analysis

STATA software IC/11.0 was used for statistical analysis. Counts were expressed in logarithmic units of microorganisms per centimetre square (log_{10} CFU/cm²), statistical analysis was performed by applying the non-parametric Mann-Whithey (or Wilcoxon) for comparison of means the significance level of 5%.

RESULTS

The findings show that 100% of the average daily log belong to the NS (Not satisfactory) class for both APC (6.16 ± 0.17 log_{10} CFU/cm²) and enteric bacteria count (4.62 ± 0.16 log_{10} CFU/cm²), with the presence of *Salmonella* in 90% of the analysed carcasses (Table 1). The daily level of APC and enteric bacteria counts varied according to sampled sites and sampling days (Table 2). With respect to the acceptable levels defined by EC standards (N° 2073/2005) none of the aforementioned results is satisfactory.[14]

The average of APC did not change from one site to another. However, a significant variation at 95% level of confidence was observed between the enteric bacteria counts of the neck and the thigh (Table 3).

Table 4 gives an overview of the overall degree of contamination per sites. It reveals that the thigh was the most contaminated part during the slaughtering process, with 100% unsatisfactory results for APC and enteric bacteria count together with a high presence of *Salmonella* (75%). The thigh is followed by the shoulder, with 100% unsatisfactory for APC, enteric bacteria count and *Salmonella* in 55% of samples. These two sites are followed respectively the neck and flank.

Table 1: Average daily log according to sampling days.

Days	APC (log_{10} CFU/cm^2) Avg ± SD	Class	Enteric bacteria count (log_{10} CFU/cm^2) Avg ± SD	Class	*Salmonella* (presence or absence)	Class
D 1	6.26a ± 1.33	NS	5.01a ± 0.59	NS	100% present	NS
D 2	6.51a ± 1.54	NS	4.76ab ± 1.78	NS	60% present	NS
D 3	5.00b ± 1.33	NS	4.19b ± 0.64	NS	100% present	NS
D 4	6.77a ± 1.39	NS	4.35ab ± 1.39	NS	100% present	NS
Standard	3.5 ≤ A < 5 S NS		1.5 ≤ A < 2.5 S NS		Absence	

Avg: Average; SD: Standard deviation; NS: Not satisfactory; S: Satisfactory; A: Acceptable; D: Day. Mean in the same column, followed by different letters differ significantly at 5%

Table 2: Daily variation of the APC and enteric bacteria counts between sampled sites (carcass parts).

Sampling days	Site	Average (log_{10} CFU/cm^2) ± SD	Class	Average (log_{10} CFU/cm^2) ± SD	Class
D 1	Neck	6.92 ± 0.62	NS	5.20 ± 0	NS
	Shoulder	6.66 ± 0.74	NS	5.20 ± 0	NS
	Flank	5.30 ± 2.13	NS	4.60 ± 1.20	NS
	Thigh	6.18 ± 1.05	NS	4.93 ± 0.37	NS
D 2	Neck	6.18 ± 1.11	NS	5.55 ± 0.51	NS
	Shoulder	7.12 ± 1.16	NS	3.94 ± 2.26	NS
	Flank	6.84 ± 2.45	NS	6.20 ± 1.44	NS
	Thigh	5.90 ± 1.23	NS	3.52 ± 0.97	NS
D 3	Neck	4.65 ± 0.85	A	4.20 ± 1.27	NS
	Shoulder	5.22 ± 0.77	NS	4.65 ± 0.77	NS
	Flank	4.90 ± 1.54	A	4.10 ± 0.26	NS
	Thigh	5.22 ± 1.98	NS	3.96 ± 0.63	NS
D 4	Neck	6.52 ± 0.38	NS	4.28 ± 1.31	NS
	Shoulder	7.02 ± 1.80	NS	4.17 ± 1.74	NS
	Flank	7.10 ± 1.19	NS	3.88 ± 1.22	NS
	Thigh	6.46 ± 2.01	NS	5.06 ± 1.53	NS

SD: Standard deviation; NS: Not satisfactory; A: Acceptable; D: Day

Table 3: Variation of the average counts between the four sites on the 20 carcass.

Sites	Parameters		
	APC Average ± SD log_{10} CFU/cm^2	Enteric bacteria count Average ± SD log_{10} CFU/cm^2	*Salmonella* (presence)
Neck (A)	6.14a ± 1.10	4.87a ± 0.98	70%
Shoulder (B)	6.24a ± 1.96	3.58ab ± 2.28	55%
Flank (C)	6.03a ± 1.99	4.05ab ± 2.20	60%
Thigh (D)	5.94a ± 1.56	3.48b ± 2.08	75%

SD: Standard deviation. Mean in the same column, followed by different letters differ significantly at 5%

Table 4: Summary of results per site for the three parameters.

Site	Parameters			
	Classification	APC (%)	Enteric bacteria count (%)	*Salmonella* (%)
Neck	A	25	0	30
	NS	75	100	70
Shoulder	A	0	0	45
	NS	100	100	55
Flank	A	25	0	40
	NS	75	100	60
Thigh	A	0	0	25
	NS	100	100	75

A: Acceptable; NS: Unsatisfactory

DISCUSSION

Global contamination

Aerobic plate count is one of the criteria of carcass contamination and an indicator of the hygiene status of the slaughtering process according to the EC Regulation N° 2073/2005.[14] The average level of contamination of cattle carcasses in this study was 6.16 $\log_{10}$ CFU/cm^2; this value indicates a relatively high level of contamination. It is much higher than that found by Salifou et al. who obtained an average of 3 $\log_{10}$ CFU/cm^2 over six weeks of sampling in the same slaughterhouse.[11] Nevertheless, the average reported by Salifou et al. is similar to that obtained by Collobert from 233 cattle carcasses in four abattoirs of Calvados where the average contamination was 3.78 $\log_{10}$ CFU/cm^2.[4,11] Siham et al. obtained at El-Harrach slaughterhouse in Algeria an average of 3.11 $\log_{10}$ CFU/cm^2.[20] Several authors reported similar values to that of Siham et al., including Phillips et al. and Zweifel and Stephan who obtained respective values of 3.33 $\log_{10}$ CFU/cm^2 and 3 $\log_{10}$ CFU/cm^2.[21,22] Likewise, the current results are far higher than those of Summer et al., Phillips et al. in Australia who reported respective values of 2.59 and 2.28 $\log_{10}$ CFU/cm^2.[23,24]

Moreover, the average of 6.16 $\log_{10}$ CFU/cm^2 is slightly greater than that obtained by Dennaï et al. and El-Hadef et al. who obtained respectively 5.15 $\log_{10}$ CFU/cm^2 in 32 carcasses sampled at the municipal slaughterhouse of Kenitra in Morocco and 5.34 $\log_{10}$ CFU/cm^2 at the slaughterhouse of Constantine.[3,25] Similar observation is recorded in relation to the average of Salifou et al., 5.99 $\log_{10}$ CFU/cm^2 in 60 carcasses obtained from slaughterhouses of Cotonou/Porto -Novo.[12]

The interpretation of this result with respect to EC Regulation 2073/2005 shows that it is unsatisfactory.[14] This result can be explained by the existence of multiple sources of contamination, such as contact between carcasses and contaminated tools or operators' hands. The level of external cleanliness of animals before slaughtering also affects the carcass contamination level. For instance, a study by Evoy et al. showed that there was a difference of contamination for the total surface flora of carcasses from externally clean animals and much dirtier ones.[26] This explanation was further confirmed by Vallotton.[5]

The nonparametric test used for comparison of means revealed a significant difference between the 1st and 3rd day, 2nd and 3rd day, then the 3rd and 4th day. The interpretation of these results shows that the third day is the least contaminated. This could be explained by the fact that a general cleaning of the slaughterhouse was made a week earlier. The application of a correct cleaning method therefore contributes to the reduction of carcasses contamination.

With regard to surface contamination of carcasses by enteric bacteria, it is obtained a relatively high average of 4.62 $\log_{10}$ CFU/cm^2. This result is significantly higher than that found by who obtained the respective average counts of 1.2 $\log_{10}$ CFU/cm^2 from slaughterhouses of Cotonou/Porto-Novo, 2.16 $\log_{10}$ CFU/cm^2 bovine carcasses in Algeria, 1.2 $\log_{10}$ CFU/cm^2 in France and 1.42 $\log_{10}$ CFU/cm^2 233 cattle carcasses slaughtered in four slaughterhouses Calvados.[4,5,11,27] El-Hadef et al. obtained a relatively lower average of 1.39 $\log_{10}$ CFU/cm^2 for the enumeration of fecal coliforms.[25] However, the average count found in the present study is less than the one reported by Salifou et al. who obtained 5.14 $\log_{10}$ CFU/cm^2 on six days sampling in the slaughterhouse of Cotonou/Porto-Novo.[12]

The hygiene of the slaughtering process is also unsatisfactory regarding the enteric bacteria load. This could be explained by the existence of various sources of contamination within the slaughterhouse. Poor evisceration practices that often lead to rupture of the gastrointestinal tract. Together with issues like non-ligation of the rectum and the oesophagus, poor handling practices during skin removal, poor personnel hygiene and the level of cleanliness of animals before slaughtering are many sources of contamination that could justify these results. Comparison of the average enteric bacteria counts between the different sampling days also revealed a significant difference between days 1 and 3. As for the total flora day 3 has a lower contamination rate than the other days.

Of the level of contamination by *Salmonella* recorded in this study (60 to 100% each sampling day) are higher than those reported by other studies. These results are alarming, as several authors have shown in their studies a total absence of *Salmonella* on the surface of carcasses. For example, Phillips et al. reported that no *Salmonella* isolate was recovered on any of the 1117 sheep carcasses tested and similar observations were reported by

Bhandare et al. and Dennaï et al.[3,21,28] Likewise, Salifou et al. reported a total absence of such pathogenic microorganisms from beef carcasses that they have analysed in Cotonou/Porto-Novo slaughterhouses.[11] Besides, recent studies conducted in South Africa by Nicoline F. et al. revealed that despite detection of *E. coli* (67.5%) and *S. aureus* (32.5%) on the carcasses of beef and pork, *Salmonella* was absent in all analysed samples.[29] Althought, other researchers like Siham et al. isolated *Salmonella* from the surface of carcasses, it was in a relatively lower concentrations (one positive out of 90 samples).[20] Sierra et al. and Small et al. reported *Salmonella* contamination levels of respectively 10% and 9.6%.[30, 31] Other authors postulated lower prevalence, these include Madden et al., Barkocy-Gallagher et al., McEvoy, et al., Rivera-Betancourt et al., and Fegan et al. and Phillips et al., who noted the levels of contamination on the surface of bovine carcasses by *Salmonella* ranging from 0% to 7.6%.[24,32-36]

Nevertheless, of the differences in the prevalence of pathogenic bacteria between this study and the aforementioned ones may be due to differences in methods, sample sizes, frequency and time of collection, transportation and storage samples, sampling seasons and age of the animals.[20]

Contamination between site

The differences between the surface bacterial loads based on anatomical sampling sites, was reported by several authors.[3,20,25,26,37] The current results show that for the total flora, the shoulder is the most contaminated site (6.24 $\log_{10}$ CFU/cm^2), followed by the neck (6.14 $\log_{10}$ CFU/cm^2). Loubamba et al. obtained a higher APC level of 5.36 $\log_{10}$ CFU/cm^2 from the shoulder being the most contaminated part in their study.[38] Also, this high contamination of the neck was reported by Zweifel and Stephan who found that the neck and chest are the most contaminated sites.[22]

The high contamination of these sites would be partly due to the fact that these two sites are part of the forequarter, which is closer to the ground after suspension of the carcass; these sites are thus exposed to the projection of dirt and contamination soil. This fact has also been reported by Siham et al., on the other hand the carcass conveying system carcass is not automatic, and workers are therefore, obliged to manually move these carcasses by carrying several of their anatomical regions including the shoulder, flank and neck.[20] This practice was also reported by Loubamba and al.[38]

However, the test of comparison of means reveals that there is no significant difference ($p>0.05$) between contaminated sites with respect to the total flora (Table 3); in fact, the sites compared in pairs gave no significant differences for this criterion.

For Enteric bacteria, the neck and the flank are the most contaminated with ones with respective counts of 4.87 and 4.05 $\log_{10}$ CFU/cm^2 per sites. Comparison of the means showed a significant difference between the neck and that of the leg (Table 3).

The thigh is the least contaminated site for both APC and enteric bacteria count, these results are consistent with that of El-Hadef et al. who showed that the thigh presented the lowest level of contamination for in sheep and cattle.[25] This is due to the distance that separates carcasses from the ground and handlers body when suspended.[20]

Unlike other authors, this study was not able to demonstrate significant differences between all the studied carcass parts as aforementioned. However, the observed contamination rates confronted with microbiological and hygiene criteria of slaughtering process revealed that the thigh was the most contaminated part, with 100% unsatisfactory results for the total flora and enteric bacteria counts with a strong presence of salmonella (present in 75% of samples of thigh). It was followed by the shoulder, with 100% unsatisfactory for the total flora, enteric bacteria and *Salmonella* in 55% of samples. Although being the site with the lowest levels of contamination, the inner part of the thigh was the site with the highest rate of dissatisfaction.

CONCLUSION

The present study entitled «Evaluation of the microbiological quality of cattle carcasses at the slaughterhouse of Cotonou/Porto-Novo» has revealed the hygienic status of the slaughtering process as well as the identification of carcasses parts that are the most exposed ones to contamination during the process. The findings demonstrate the critical hygiene status of the slaughterhouse of Cotonou/Porto-Novo. Besides, slaughterhouses are is the first link in the development of food products of animal origin, and if already at this level the safety of meat is not satisfactory; there is therefore a reason to be worried about the health of consumers. It is urgent to proceed with the complete renovation of the abattoir including the slaughtering hall, and ensure the application of good hygiene practices and the principles of HACCP.

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