P-ISSN: 2304-3075; E-ISSN: 2305-4360

International Journal of Veterinary Science

www.ijvets.com; editor@ijvets.com

Research Article

Microbial Load of Beef Sold in the Traditional Slaughterhouse and Butcher Shops in Northern Cameroon

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Article History: Received: June 14, 2015 Revised: August 21, 2015 Accepted: August 27, 2015

ABSTRACT

A classification of traditional slaughterhouses and butcher shops based on microbiological characteristics of beef was conducted in the northern Cameroon. All across this area, 6 traditional slaughterhouses and 18 traditional butcher shops were selected for the study. 125 samples were collected using the "wet and dry" method. Microbiological analyses showed significant contamination of carcasses in slaughterhouses, with average concentrations of 4.03±0.8 log cfu/cm^2; 2.26±0.8 log cfu/cm^2; 0.37±0.55 log cfu/cm^2 and 2.2±1.02 log cfu/cm^2 for mesophilic aerobic bacteria, coagulase-positive staphylococci, anaerobic sulphur-reducing bacteria and thermo tolerant coliforms, respectively. In the distribution process, bacterial densities were still increasing with average values of 4.84±1.06 log cfu/cm^2 (mesophilic aerobic bacteria); 2.95±1.1 log cfu/cm^2 (coagulase-positive staphylococci); 0.87±0.83 log cfu/cm^2 (anaerobic sulphur-reducing bacteria) and 2.83±0.97 log cfu/cm^2 (thermo tolerant coliforms). Multivariate analyses revealed that the traditional slaughterhouses in Guider, Garoua and K. Djolao produced the least contaminated carcasses compared to those in Pitoa (Garoua annex). The slaughterhouse in Manwi provided to butcher shops carcasses with high microbial densities. In the distribution process, meat from Ngaoundere and Garoua slaughterhouses was more contaminated than that coming from the slaughterhouses in Maroua, Guider and Pitoa (Garoua annex). This classification indicates that improving the hygienic quality of meat produced and distributed traditionally in the northern Cameroon depends on an utmost application of hygienic practices mainly linked to the process of production and distribution but also to the improvement of staff management involved in the different systems.

Key words: Slaughterhouse, butcher shops, traditional, beef, bacterial contamination, northern Cameroon

INTRODUCTION

In Cameroon, the beef industry is still underdeveloped. From the farm to the consumer, the structures that dominantly make up this sector remain rudimentary, with the exception of the cities of Douala and Yaounde where two modern slaughterhouses have been built since the 1980s (Mravili *et al*., 2013). In the northern Cameroon, these slaughter facilities are made up of traditional slaughterhouses. Most often, they appear in the form of sheds made of permanent and/or temporary materials covering sometimes paved surfaces sometimes associated with a rudimentary drainage systems and/or wastewater treatment systems. Moreover the transportation of carcasses to markets is characterised by the use of inappropriate means (motorcycles, bicycles, taxis, private vehicles) (Beauvilain, 1985). The traditional distribution of beef is marked by the its prolonged exposure on tables without any protection against bad weather (heat), dust and flies (Mravili *et al*., 2013). These conditions do not guarantee the production and distribution of meat safe for consumption. This raises the problem of the hygienic quality of meat in Cameroon. In fact, many studies have been carried out on the description of sanitary practices in the meat sector in developing countries such as Cameroon (Afnabi *et al*.,

Cite This Article as: Afnabi RB, RP Nameni, SS Kamdem, JJE Ngang and RB Alambedji, 2015. Microbial load of beef sold in the traditional slaughterhouse and butcher shops in Northern Cameroon. Inter J Vet Sci, 4(4): 183-189. www.ijvets.com

2014); Tanzania (Komba *et al*. 2012). Others authors have explored the microbiological load of meat produced in slaughterhouses and sold on markets in countries like Ghana (Antwi-Agyei and Maalekuu, 2014); Ethiopia (Haileselassie *et al*., 2013); Rwanda (Niyonzima *et al*., 2013). But in Cameroon, no research has yet classified the traditional systems of beef production and distribution using microbiological criteria. Indeed making a typology of meat production and distribution systems, in group of same hygiene conditions would allow to better target the sanitary actions. To achieve this goal, this study will first of all determine the level of contamination of beef carcasses in the traditional slaughterhouse and in traditional butcher shops in Northern Cameroon. Secondly, it will group into classes these systems of production and distribution of beef.

MATERIALS AND METHODS

Sampling

The study was conducted in five major cities in the Northern part of Cameroon, from March to June 2014. Samples were collected from Maroua, Guider, Pitoa, Garoua and Ngaoundere. Altogether 125 samples were collected in slaughterhouses (71) and butcher shops (54). Three sampling periods were chosen in the respective sites and between 4 and 5 samples collected per period and per site (Table 1). The choice of slaughterhouses and butcher shops was made on the base of a cluster analysis performed in a previous study (Afnabi *et al*., 2014). The selection of carcasses for sampling was randomly done in the slaughterhouses. Sampling was performed following the "Wet and Dry" technique (McEvoy *et al*., 2004). At the slaughterhouse, the sampling were always taken immediately after the veterinary inspection, while at the level of butcher shops they were always taken between 9:30 AM and 10:30 AM, that is, just after the cutting operations. The samples were kept between 0 and 4 ° C in a cool box containing ice packs (ICECATH® of eutecma gmbh). The collected samples were immediately transported to the National Veterinary Laboratory in Garoua (Cameroon) for analysis.

Microbial analysis

The samples were all analysed within 24 hours after sampling. The techniques and bacteria sought in this study were consistent with counts on petri dish of the total aerobic bacteria (TVC= total viable aerobic count) (AFNOR, 2013), thermo tolerant coliforms (TTC) (AFNOR, 2009b), anaerobic sulphur-reducing (ASR) bacteria (AFNOR, 2009a) and coagulase-positive staphylococci (CPS) (AFNOR, 2004).

Data analysis

Results in cfu/cm^2 were expressed in logarithm to base 10 (Log 10). For analyses that did not identify bacteria, the number 1 was added to all cfu/cm^2 of this phase to facilitate the conversion into Log 10. A Principal component analysis (PCA) and a cluster analysis (CA) were carried out for the production and distribution of meat, using the SPAD 5.5 software. A Canonical Variate Analysis (CVA) was done using the XLSTAT Stat Pro 7.5 software. The following model was developed to highlight the variables that best separate the classes identified in CA.

$$\text{Class} = \text{Constant} + a*\text{TTC} + b*\text{CPS} + c*\text{ASR} + d*\text{TVC}$$

TTC, CPS, ASR and TVC are the respective contamination level of meat expressed in log cfu/cm^2. The constant and the parameters a, b, c and d will be determined by CVA analysis. Decision N°2001/471/EC of the European Union (European Communities Commission, 2001) was used for the comparison of microbial densities obtained in this study with respect to the standards of the European slaughterhouses.

RESULTS

Below are the results of the study being conducted in slaughterhouses and butcher shops.

Microbial contamination of meat in slaughterhouses

Of all the slaughterhouses surveyed, the microbiological contamination of carcasses ranged from 2.41 to 5.97 log cfu/cm^2 (Table 2). The TVC was strongly represented with an average of 4.03 log cfu/cm^2 compared to ASR, which had the lowest average observed (0.37 log cfu/cm^2). The prevalence of TTC, of CPS and TVC exceeded 94% while that of ASR was around 41%.

Multivariate analyses were performed on the levels of contamination of beef from traditional production systems. PCA has brought to light two main axes that explained 52.18% (axis 1) and 21.55% (axis 2) of correlations between the levels of beef contamination. At factor 1, bacterial loads of carcasses from slaughterhouses in Ngaoundere area (Ngaoundere 7, 8 and 9) were inversely correlated to those of Garoua area (Pitoa 1, 2 and 5) with respective contributions (ctbr) of 16.48% and 13.5% to the formation of this axis (Figure 1). For axis 2, a negative correlation was observed between the meat contamination levels of Maroua (Maroua 1, 2 and 3; ctbr: 16.1%) and Guider (Guider 9, 10, 12 and 15; ctbr: 13.5%) city slaughterhouses. PCA has also designated axis 1 as a size factor, because all variables indicating contamination of meat were positively correlated (Figure 1). The total aerobic bacteria showed a strong affinity with all other bacteria ($r = 0.19$, $p < 0.05$)

The storage of meat carcasses according to their bacterial load revealed three classes (Figure 1). The first (class 1/3; 30 carcasses) comprised 30% of carcasses from Guider, 26% from Maroua and 23% Garoua. The second class (2/3; 27 carcasses) consisted of cattle slaughtered in slaughterhouses in Pitoa (38%), in Guider (18%), in Garoua (18%), and in Ngaoundere (18%). The third group (3/3) had 14 carcasses with 36%, 28% and 21% respectively from Ngaoundere, Maroua and Garoua slaughterhouses.

The search of parameters that will help to best separate the classes identified by CA has enabled us to differentiate two main factorial discriminant axes. With a contribution of 79.6% of the total variance, axis 1 (Table 3) will mainly describe the relationship between the variables involved in the differentiation of classes observed previously. The participation of aerobic bacteria in the formation of axis 1, ranged from 0.56 to 0.84 for

Table 1: Slaughterhouses and butcheries sampled

Cities	Municipal Slaughterhouses	Number of carcasses sampled per slaughterhouse			Total number of sampled carcasses	Nomber of sampled butcher shops			Total number of sampled butcher shops
		First Phase	Second Phase	Third Phase		First Phase	Second Phase	Third Phase	
Maroua	Maroua (Kongola Djolao)	5	5	5	15	4	4	4	12
Guider	Guider	5	5	5	15	4	4	4	12
Pitoa	Pitoa (Garoua annex)	5	5	5	15	3	3	3	9
Garoua	Garoua	5	5	5	15	3	3	3	9
Ngaoundere	Ngaoundere (Manwi)	3	4	4	11	4	4	4	12
	Total per phase	23	24	24	71	18	18	18	54

Table 2: Carcasses contamination at the slaughterhouses

Germs of contamination	Beef carcasses	Prevalence (%)	Mean ($\log_{10}$ ufc/cm^2)	Standard deviation	Minimum $\log_{10}$ (ufc/cm^2)	Maximum ($\log_{10}$ ufc/cm^2)
TTC	71	94.36	2.2	1.02	0.00	4.10
CPS	71	98.59	2.26	0.81	0.22	4.36
ASR	71	40.84	0.37	0.55	0.00	1.97
TVC	71	100	4.03	0.80	2.41	5.97

Table 3: CVA synthesis for the meat production system

			Coordinates of variables			Coefficient of the canonical discriminant function		
	F1	F2	Variables	F1	F2	Variables	F1	F2
Eigen values	2.668	0.684	TTC	0.745	0.588	TTC	0.841	2.013
% of variance	79.601	20.399	CPS	0.560	-0.640	CPS	0.415	1.045
Cumulative %	79.601	100.000	ASR	0.673	-0.045	ASR	1.082	-0.933
			TVC	0.845	-0.348	TVC	0.934	0.007
						Constant	-6.962	-0.547

Table 4: Contamination of beefs in the traditional butcher's shops

Germs of contamination	Butcher shops	Prevalence (%)	Mean ($\log_{10}$ ufc/cm^2)	Standard deviation	Minimum ($\log_{10}$ ufc/cm^2)	Maximum ($\log_{10}$ ufc/cm^2)
TTC	54	100	2.83	0.97	0.97	5.10
CPS	54	100	2.95	1.10	0.10	5.26
ASR	54	70.37	0.87	0.83	0.00	3.03
TVC	54	100	4.84	1.06	2.80	7.27

Table 5: CVA synthesis for beef distribution system

		Coordinates of variables		Coefficient of the canonical discriminant function	
	F1	Variables	F1	Variables	F1
Eigen value	2.481	TTC	0.781	TTC	0.156
% of variance	100	CPS	0.701	CPS	0.301
Cumulative %	100	ASR	0.947	ASR	1.351
		TVC	0.871	TVC	0.447
				Constant	-4.665

staphylococci total viable count. On axis 2, coliforms and staphylococci were different with respective shares of 0.58 and -0.64 (Table 3). The standardized canonical discriminant function helped define the factorial discriminant axes but also the assignment function (the score function) of individuals in the new classes (1/3, 2/3 and 3/3). The score function appeared as follows:

Class 1/3 = -23,75 + 1,87 TTC+ 2,08 CPS - 0,21 ASR + 11,13 TVC
Class 2/3 = -35,93 + 5,24 TTC + 1,71 CPS + 2,23 ASR + 12,47 TVC
Class 3/3 = -57,036 + 4,74 TTC + 4,39 CPS + 4,3 ASR + 15,38 TVC

Microbial contamination of meat at butchers shops

The traditional distribution of beef was marked by high contamination of aerobic bacteria whose bacterial load ranged from 2.8 to 7.27 log cfu/cm^2. ASR bacteria were less represented with values ranging between 0 and 3.03 log cfu/cm^2. No matter where the butcher shops were located, staphylococci, coliforms and aerobic bacteria have been found on meat (100% prevalence), while ASR were only detected in 70% of cases (Table 4).

The analysis of bacterial densities in meat in traditional butcher shops revealed a significant positive correlation between all the bacteria studied. CPS had lower correlations compared to TTC (r = 0.42; P<5%) and ASR (r = 0.49; P<5%). PCA revealed a close relationship between the contamination of meat from butcher shops in Ngaoundere and Pitoa (Figure 3), with respective contributions of 37.5% (Hamid_Ndr 1, 2, and 3; Souley 3; Hassan 2, and 3) and 10% (Abdou (Moussa) 3) on the formation of axis 1. On the same axis, the bacterial loads of meat were positively correlated between the butcher shops of Maroua (Ctbr: 13.8%; Ou 1 and 3; Hamad 1 and 3; Lam 1) and Garoua (ctbr: 10%; Gondji (Babai) 1; Bouba1) (Figure 3). Axis 1 has been mainly described because it showed 72% of the correlations between the studied variables.

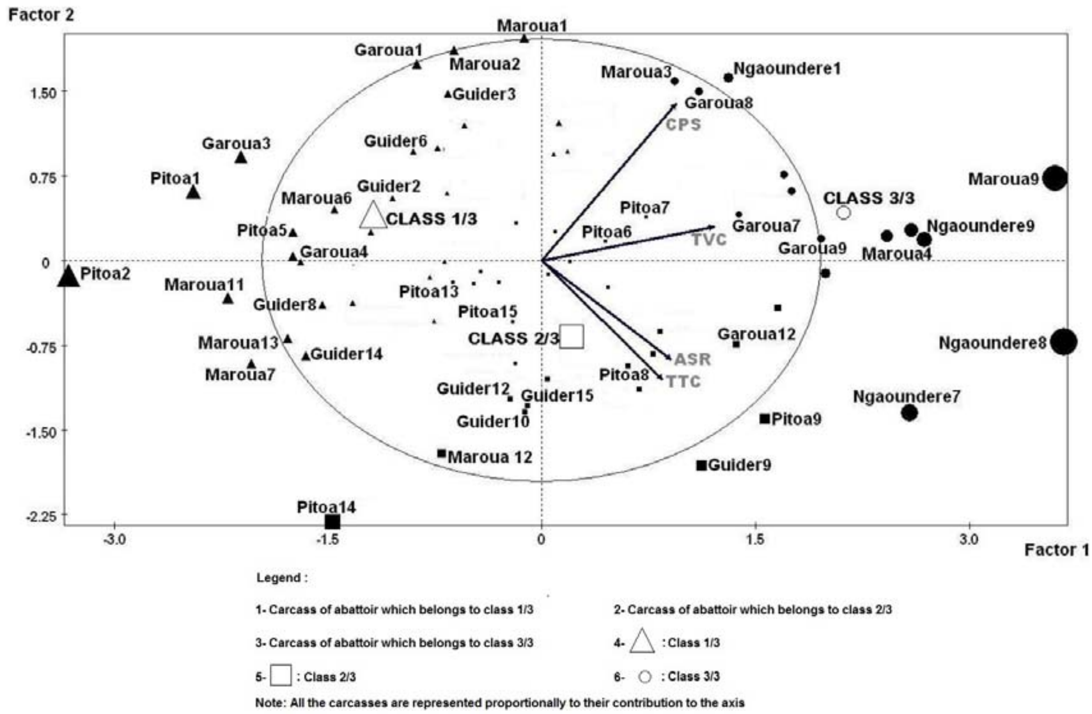

Fig. 1: PCA of beef microbial contamination in traditional abattoirs

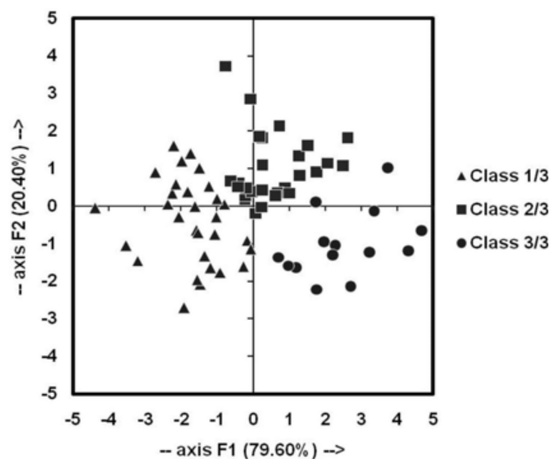

Fig. 2: Representation of slaughterhouses' meat classes after CVA

CA revealed two main classes (Figure 3). Meat from Maroua, Guider and Pitoa made up the first class (1/2) in respective proportions of 32.4%; 26.5% and 20.5%. 2.2 Class 2/2 was composed of meat from butcher shops from Ngaoundere (45%) and Garoua (25%). 34 samples of meat from 17 butcher shops were associated with class 1/2. For class 2/2, there were 12 butcher shops involved and 20 samples of meat.

The CVA showed that axis 1 was the determining factor of class distinction from CA (contribution to variance = 100%). The bacteria recorded on this meat were involved in the formation of this axis by their location which ranged from 0.7 for CPS to 0.94 for ASR. CVA has provided the coefficients for the canonical discriminant function (Table 5), which helped develop the model of the ranking function (score function) as shown below:

Class 1/2 = -19,22 + 2,86 TTC - 0,01 CPS -3,55 ASR + 7,57 TVC

Class 2/2 = -36,01+ 3,36 TTC + 0,95 CPS + 0,77 ASR + 9 TVC

DISCUSSION

The mean concentrations of TVC in slaughterhouses (4.03 log cfu/cm^2) and butcher shops (4.84 log cfu/cm^2) were greater than or equal to the limit (4.3 log cfu/cm^2) set by Decision No 2001/471/EC of the European Union (European Communities Commission, 2001). The average contamination of beef in slaughterhouses and markets by TTC (2.2 and 2.83 log cfu/cm^2), ASR (0.37 and 0.87 log cfu/cm^2) and CPS (2.263 and 2.95 log cfu/cm^2) that are associated with the values of TVC were observed. Similar results were reported by Salifou *et al*. (2013) in his study on the evaluation of the quality of beef in Benin (West Africa) but also by Nouichi and Hamdi (2009) which noted concentrations of 4.48 and 2.92 log cfu/cm^2, in aerobic bacteria and faecal coliforms respectively in El-Harrach (Algeria). These levels of contamination of beef are mainly explained by the non-observance of hygienic measures observed both in the production, distribution and sale of meat (Obeng *et al*., 2013; Haileselassie *et al*., 2013). As a matter of fact in all surveyed slaughterhouses and butcher shops, serious problems were encountered including a poor application of the principles of cleaning and disinfection; a severe lack of supervision of staff on hygiene; old, inadequate, non-existent infrastructure, etc. Also, the assessment of the level of bacteriological contamination of this study shows a growing evolution of bacterial densities from the slaughterhouses to the butcher shops. This observation was also reported by Nyonzima *et al*. (2013) who explain this phenomenon by the accumulation

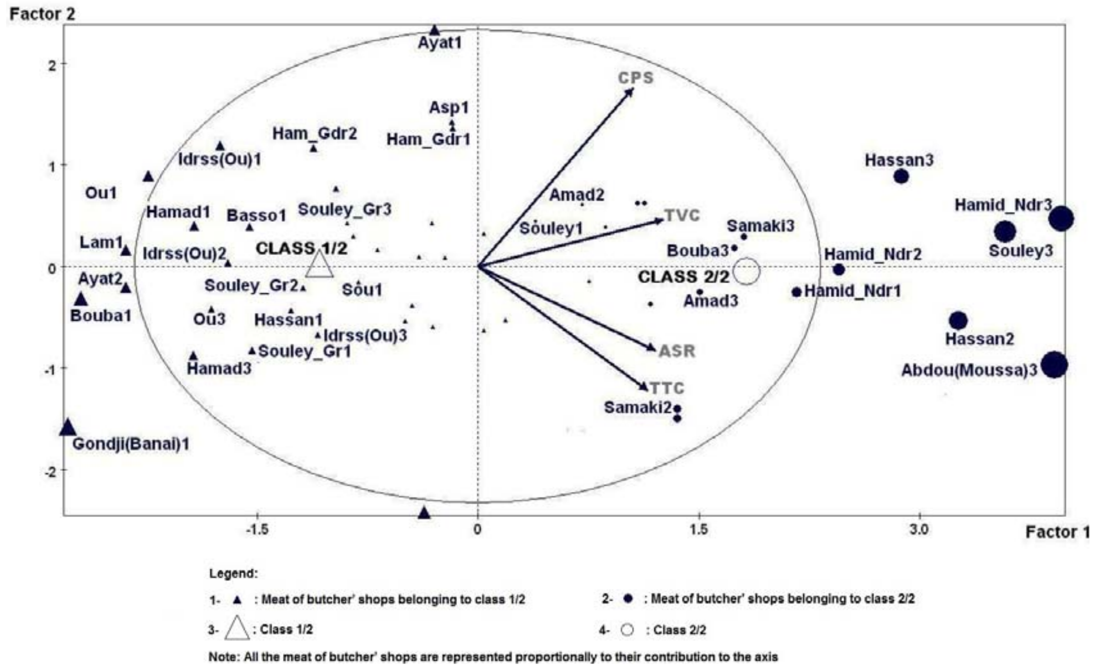

Fig. 3: PCA of beef microbial contamination in traditional butcher shops.

of poor hygienic practices in the transportation and marketing of beef.

The difference described in the PCA between microbial densities of carcasses from Ngaoundere and Garoua shows a contrast between a production system of less contaminated meat (Garoua) to contaminated meat (Ngaoundere). Axis 2 of this analysis confronted products from slaughterhouses in Maroua and Guider, which showed intermediate degrees of contamination with respect to axis 1. CA and CVA will confirm these trends by identifying three main classes of carcasses, notably class 1/3 consisting of meat with low microbial loads (TVC = 3.51±0.6 log cfu/cm^2) compared to class 2/3 with intermediate densities (TVC = 4.02±0.4 log cfu/cm^2) and class 3/3 with the highest concentrations (TVC = 5.17±0.5 log cfu/cm^2).

The strong involvement of TVC to the formation of the discriminant function and the positive correlations observed between this flora and the others bacteria attest the use of TVC as the determining factor in the characterisation of traditional production systems of meat. This is explained by the fact that the sources of contamination by mesophilic aerobic vary widely (the environment, the flora of the gastro-intestinal tract of the animal, cross-contamination of carcasses, incorrect handling) (Ghafir and Daube, 2007). In addition, samples were collected at the end of the production process but many authors (Jericho *et al*., 1992) showed that such samples do not determine the origin or cause of contamination of meat. Considering the assessment scale proposed by Decision No 2001/471/EC of the European Union, the contamination of carcasses from 1/3 can be considered acceptable (density scale between 2.8 - 4.3 log cfu/cm^2). Slaughterhouses associated with class 1/3 (slaughterhouses of Guider, Garoua and Djolao) were all different. But some practices from these slaughterhouses have helped to reduce the bacterial densities of their beef carcasses. In fact, the slaughterhouse of Garoua distinguished itself by a mechanised load transfer combined with a better organisation of work (separation of healthy and contaminated areas, good sanitary inspection). Those of Guider and Djolao were characterised by the presence of fixed hooks for hanging carcasses, plus a showering of carcasses before veterinary sanitary inspection (Afnabi *et al*., 2014). These characteristics of the Garoua slaughterhouse are in line with certain recommendations in the code of hygienic practice for meat hygiene of the *Codex Alimentarius* (FAO/WHO, 2003). The peculiarity of the slaughterhouses of Guider and Djolao is confirmed by Gorman *et al*. (1995) in his study on the effect of showering on reducing the contamination of beef carcasses. CVA revealed a significant contribution of staphylococci (-0.64) to the formation of the discriminant axis 2. This was confirmed between class 2/3 that was different by the average load of its carcasses (CPS 1.99±0.5 log cfu/cm^2; TTC: 2.9±0, 7 log cfu/cm^2) and class 3/3 (CPS: 3.29±0, 4 log cfu/cm^2; TTC: 2.73±0.7 log cfu/cm^2) (Figure 2). Carcasses from the slaughterhouse of Pitoa (Garoua Annex) named class 2/3 while those of the slaughterhouse of Manwi reflected class 3/3. The difference in concentration in staphylococci between the two structures may be attributed to the ratio between the number of the meat handlers (Pitoa 30 and Mamwi: 16 assistant butchers) and the number of animals slaughtered per day (Pitoa: 25 and 4 animals per day).

The dissimilarities noted in axis 1, combined with the results of CA show that butchers who got their meat from the municipal slaughterhouses of Ngaoundere and Garoua were less clean (class 2/2: bad system). However, those who got their meat from slaughterhouses in Maroua (municipal slaughterhouses in Maroua, Makabai and Kongola Djolao) in Guider and Pitoa (Garoua annex) belonged to a better distribution system for beef (class

1/2). The combination of significant correlations between all the variables considered and the participation (0.7 to 0.9) of these to the separation of classes demonstrate the key role played by the bacterial concentrations in the characterisation of the traditional distribution of beef (Ghafir and Daube, 2007). Though having low bacterial levels compared to class 2/2 (TVC = 5.86±0.7 log cfu/cm^2), class 1/2 has an average density in TVC (4.24±0.6 log cfu/cm^2), at the upper limit of the value characterising the acceptability of the meat. This allows to state that meat of this group was generally unacceptable. With the classification of the slaughterhouse of Garoua among the cleanest, logic would have placed meat from this slaughterhouse among the less dirty for sale, as practices related to transportation of meat from the slaughterhouse were similar. However, the opposite was observed. The parameter that could explain the type of butchers corresponding to class 2/2 is the large number of staff (≥ three) who handled the meat in the butcher shops concerned. The issue of the impact of food handling on microbiological contamination has been reported by several authors in developing countries (Abd-Elaleem *et al*., 2014; Niyonzima *et al*., 2013) as in developed countries (Da Cunha *et al*., 2014; Gomes-Neves *et al*., 2011). The direct consequence of this practice is reflected in class 2/2 through the high levels of TTC (3, 66±0.8 log cfu/cm^2) and CPS (3.80±0.8 log cfu/cm^2) on meat. The illustration of this observation was made by several studies (Koffy-Névry *et al*., 2011; Iroha *et al*., 2011) which showed an increase of these bacteria in the traditional distribution of beef in developing countries.

Conclusion

The analysis of bacterial contamination of meat in this study shows that, the reduction in bacterial densities in slaughterhouses and traditional sale of beef go through an utmost application of hygienic practices mainly linked to the process of production and distribution but also to the improvement of staff management involved in the different systems.

Acknowledgement

We would like to be grateful to Dr Stanley Fon Tebug and to the staff of the national veterinary lab (LANAVET) for their collaboration.

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