

ASSESSMENT OF *SALMONELLA*, *ESCHERICHIA COLI*, *ENTEROBACTERIACEAE* AND AEROBIC COLONY COUNTS CONTAMINATION LEVELS DURING THE BEEF SLAUGHTER PROCESS

F. NYAMAKWERE¹, V. MUCHENJE^{1,4}, B. MUSHONGA², M. MAKEPE³ and G. MUTERO³

¹Department of Livestock and Pasture Science, University of Fort Hare, Alice, South Africa

²Department of Veterinary Medicine, University of Namibia, Windhoek, Namibia

³Veterinary Laboratory, Department of Rural Development and Agrarian Reform, Grahamstown, South Africa

⁴Corresponding author.

TEL: WORK: +27-40-602-2059,

FAX: +27 86 628 2967;

MOBILE: +27844221487;

EMAIL: vmuchenje@ufh.ac.za

Received for Publication October 23, 2015

Accepted for Publication January 25, 2016

doi: 10.1111/jfs.12275

ABSTRACT

The study was conducted to determine the level of *Salmonella*, *Escherichia coli*, *Enterobacteriaceae* and aerobic colony counts (ACCs) contamination in beef carcass, equipment, slaughtermen hands and water at selected stages in abattoirs. Cattle carcasses were sampled at four sites (rump, neck, flank and brisket) from a low throughput (LTA) ($n = 240$) and high throughput (HTA) ($n = 384$) abattoirs. Using conventional biochemical tests, HTA yielded significantly higher ACC ($5.2 \log \text{CFU/cm}^2$), *E. coli* ($2.6 \log \text{CFU/cm}^2$) and *Enterobacteriaceae* ($2.9 \log \text{CFU/cm}^2$) mean scores after skinning and evisceration. Washing and chilling did not cause any significant ($P > 0.05$) changes in bacterial counts. This implies use of non-potable water and poor chilling methods. *Salmonella* was not detected on all sampled carcasses. Overall, slaughtermen hands and equipment in the dirty area yielded more bacterial counts compared to clean area from both abattoirs. Therefore, these findings show that the equipment, slaughtermen hands and water are sources of contamination during the slaughter process.

PRACTICAL APPLICATIONS

Microbiological analyses of beef slaughtering process will help identify possible modes of contamination. This will help the responsible authorities to take appropriate steps to improve meat safety in abattoirs. Currently, the Department of Agriculture, Forestry and Fisheries, Sub-Directorate Veterinary Public Health of South Africa, do not have a meat Microbiological Monitoring Program. Meat inspection is based mainly on visual assessment for cleanliness, of which this cannot detect the most important foodborne hazards such as *E. coli*, *Salmonella* spp and *Campylobacter*. Therefore, there is a need to develop more effective meat inspection programs in order to prevent contamination, foodborne illnesses and deaths. Findings from this study can also act as a the foundation for the development of acceptable microbiological standards or guidelines for abattoirs in South Africa and other developing countries as well as help in the development of more effective meat inspection programs.

INTRODUCTION

Beef contains approximately 70–73% water, 20–22% protein and 4.8% lipids (Alan *et al.* 1995; Hudson *et al.* 1996). This

chemical composition predisposes the product to microbiological contamination if appropriate hygiene practices are not followed (Nel *et al.* 2004; Abd-Elaleem *et al.* 2014).

Consequently, abattoirs and other meat processing plants have to adhere to proper hygiene practices during the slaughter process including risk-based prevention strategies in order to assure consumer health protection and meat quality. Risk-based prevention measures include the implementation of proper hygiene assessment programs as addressed by the Hazard Analysis and Critical Control Program (HACCP) systems. These systems are reported to be very effective in controlling and preventing food contamination during slaughter and processing (Milius *et al.* 2012). For the proper assessment and evaluation of these hygiene systems, it is very important to rely on indicator microbiological data since carcasses might be contaminated even though they may appear to be visually clean (Gill 2003; Lasok and Tenhagen 2013; Milius *et al.* 2014). Visual assessment cannot detect the most important foodborne microorganisms like *Escherichia coli*, *Salmonella spp*, *Listeria spp* and *Campylobacter* (Hill *et al.* 2013). These organisms have been used as indicators of possible pre- and post-slaughter contamination (Bello *et al.* 2011; Niyonzima *et al.* 2013).

The starting source of contamination reported is the animal skin and also during evisceration, pluck removal and trimming (Gill *et al.* 2003; Govender *et al.* 2013). During skinning and evisceration, bacteria especially those responsible for foodborne diseases can be transferred from initial sources to the carcasses. On the other hand, Buncic (2006) reported that healthy animals can still be carriers of pathogenic bacteria. Therefore, hides and gut contents need to be removed properly in order to reduce chances of cross contamination. The bacterial load is expected to decrease from skinning up to the point of delivery after chilling. Most researches done on carcass contamination during slaughter have focused on sampling on the carcass surfaces only and this does not suggest any sources of contamination (Bello *et al.* 2011; Niyonzima *et al.* 2013; Zweifel *et al.* 2014). According to FAO (2004), most developing countries do not apply proper hygienic practices during slaughter, transportation and marketing, leading to meat microbiological contamination. Niyonzima *et al.* (2013) found that the bacterial load of beef meat sampled at different stages along the value chain were out of the European Microbiological Standards acceptable range. Moreover, Adzitey *et al.* (2011) and Hemmatinezhad *et al.* (2015) reported that most abattoirs and meat processing plants have poor hygiene quality control programs.

According to published data, there is no scientific data available in the Eastern Cape Province of South Africa for the microbiological quality of meat, equipment, slaughtermen hands and water used during the slaughter process. This information is important since the contamination of the final end product corresponds to the combined contribution of different factors and stages along the meat processing chain (Gill *et al.* 2003; Niyonzima *et al.* 2013; Zweifel

et al. 2014). Therefore, the objective of the current study was to assess the level of contamination with *Enterobacteriaceae*, *Escherichia coli*, *Salmonella spp* and aerobic colony count (ACC) of cattle carcasses at different slaughter process stages in selected abattoirs. Additionally, the quantitative relationship between the cattle carcass, slaughtermen hands, equipment and water microbiological quality was also assessed.

MATERIALS AND METHODS

Abattoir and Slaughter Processes

This study was carried out in two selected abattoirs which are, Grahamstown and East London from the Eastern Cape Province of South Africa. The abattoirs were classified into two major categories which are low throughput abattoir (Grahamstown: LTA) and high throughput abattoir (East London: HTA). Both abattoirs are approved by the competent authorities. The LTA slaughters ~18 animal units per day. All animal species use the same slaughter floor. The HTA slaughters ~165 animal units per day. However, different slaughter floors are used for each animal species.

The slaughter lines in both abattoirs are divided into two major sections which are the dirty and clean areas. Stunning, exsanguination, skinning, head and feet removal are done in the dirty area and then moved to the clean area for evisceration. The carcass is split along the midline with a splitting saw, trimmed during meat inspection and then washed using cold water to remove visual debris. Before chilling, the carcass is weighed, graded and stamped. The operations are almost the same as those described by Zweifel *et al.* (2014). Both abattoirs chill the carcasses for ~16–20 h at 5–7°C before distribution to respective outlets.

Sampling

The number of carcasses to be sampled per day were calculated according to Food Safety and Inspection Service (FSIS) Directive 6420.2, Livestock Carcass Examination (United States Department of Agriculture, 2011). This technique depends on the number of animals slaughtered in each abattoir per day as shown in Table 1. Bovine carcass units were selected randomly. On the other hand, the sampling sites

TABLE 1. DETERMINATION OF THE NUMBER OF CARCASSES TO BE SAMPLED IN EACH ABATTOIR

Number of animals slaughtered per day	Number of carcass units to examine
100 or fewer	2
102–250	4
251–500	7
More than 500	11

Adopted from USDA (2011)

were selected according to ISO 17604 (2003) and European Union (EU) Directive 2001/471/EC guidelines (DAFF, 2010). A total of 100 cm² was swabbed from four sites forming a pooled sample for each carcass (neck, brisket, flank and ramp). The carcasses were sampled at different selected stages during slaughter, that is, after skinning, evisceration, washing and chilling when the carcass was ready for delivery. For the LTA a total of 240 samples were collected at different stages from 60 different carcasses and 384 samples for HTA from 96 carcasses. Data were collected during a six month period from November 2014 to April 2015.

The swab technique was used for sample collection; this procedure is non-destructive and is used to collect carcass surface samples with Swab Rinse Kit (SRK) foam spatulas for microbiological analysis. Sterile gloves were used all the time and were changed between carcasses. At each site, a moistened swab (NaCl peptone solution) was wiped both in the horizontal and vertical direction across the sampling site (100 cm²) for 30 s. Samples were packed and labeled according to the corresponding carcass number, site, town of collection and abattoir name. The samples were then transported to the laboratory on the same day for bacterial analysis in cooler boxes to prevent microbial growth. The packages were kept intact until they were aseptically opened in the laboratory for examination. Samples were analyzed for ACC, *Enterobacteriaceae*, *Escherichia coli* and *Salmonella* spp.

Microbiological Analyses

Aerobic Colony Counts and *Enterobacteriaceae*. ACCs and *Enterobacteriaceae* are an indication of overall microbial contamination of food and higher counts indicates poor hygiene. For the enumeration of ACC and *Enterobacteriaceae*, each swab sample was vortex mixed in 40 mL of 0.1% NaCl peptone solution (8.5g NaCl, 1g trypton casein-peptone, 0.1% agar and 100 mL distilled water) to make an even suspension. Tenfold serial dilution of the samples was then prepared up to 10⁻⁴ after which 1 mL was placed on to the plate count agar for ACCs enumeration. The plates were incubated for 48 h at 35C. Presumptive colonies were counted using a Colony Counter-Digital machine (3 × magnifier, White LED array, Lasec South Africa). For *Enterobacteriaceae* enumeration, 1 mL of the sample was placed on to the violet red bile glucose agar (VRBG). The plates were incubated for 24 h at 37C. Colonies seen to be pink to red or purple were selected and biochemical conformation tests were performed in accordance with the international standards (ISO 21528-2 2004).

***E. coli* Identification.** The identification of *E. coli* was done in accordance with the International Standards Guidelines, using the most probable number technique (MPN) (ISO 16649-2 2003). Thoroughly mixed the swab sample with

NaCl peptone solution then transferred 1 mL of the sample to 9 mL of the Ringer solution. Tenfold serial dilution for each swab sample for up to 10⁻⁴ were prepared and incubated at 44C for 48 h. After incubation, recorded gas production from the samples as *E. coli* positive. The MPN of *E. coli* per milliliter was determined from the Agriculture Research Council (ARC) reference tables (ARC 2010). All positive results were streaked onto McConkey agar medium for isolation and confirmation of *E. coli*. The plates were then incubated at 37C for 24 h and examined for typical smooth pinkish colonies. Transferred 0.1 mL of brilliant green broth from tubes with gas formation into 10 mL of trytone water and incubated for 48 h at 44C. Determined indole production by adding Kovac's reagent to the tryptone water.

***Salmonella* spp Identification.** The ISO international procedure was followed for *Salmonella* detection (ISO 6579 2002). Buffer peptone water (BPW) was inoculated at ambient temperature with the test swab portion and incubated at 37C for 18 h. Using a micro-pipette, 0.1 mL of the pre-enriched broth was transferred to 10 mL semi-solid Rappaport-Vassiliadis soy peptone (RVS) broth. The RVS plate was incubated at 41.5C for 24 h. The enriched solutions were streaked onto Brilliant green agar and Xylose-lysine-deoxycholate agar and incubated at 36C for 24 h. Presumptive positive results were confirmed using biochemical tests according to international standards (ISO 6579 2002).

Water Sample Collection and Analysis

Water samples were collected from the primary (tank reservoir) and secondary (taps) sources. The water was collected using 500 mL sterile glass bottles which were then aseptically sealed and labeled according to place of collection. The water samples were analyzed for the presence of overall microbial contamination including *E. coli*, *Enterococci* and coliform identification. The test procedures were performed in accordance with the International standards (ISO) guidelines using the membrane filtration method (ISO 4833 2003; ISO 21528-2 2004).

For the enumeration of ACC, 100 mL of water sample was poured onto a filter paper (pore size 0.44 µm) which trapped the bacteria. After all the water was filtered, the filter paper was then removed and placed in petri dishes containing the Plate Count Agar. This was then incubated at 35C for 48 h. *Escherichia coli* and coliform bacteria enumeration was done simultaneously; 100 mL of the water sample was filtered and then the filter paper was removed and placed in petri dishes with EMB agar. The petri dishes were incubated for 24 h at 35C. Dark-blue to violet colonies in EMB were counted as presumptive *E. coli* and salmon to red as coliforms. *E. coli* isolates were confirmed using the Indole test with Kovac's reagent. For the enumeration of *Enterococci*, the filter paper was placed in Bile Esculin agar and incubated

at 37C for 48 h. Black colonies after incubation were counted as presumptive *Enterococci*.

Contact Surface Sample Collection

Agar Contact Plate Method. Slaughtermen hands and equipment used in abattoirs also provide an indication of the hygiene and microbiological status of the products produced. The equipment's sampled include knives and saws as well as meat handlers' hands. Agar contact plates were used had an internal diameter of 5.0 cm. The dishes had a contact surface of 20 cm², filled with violet red bile glucose agar and the others with plate count agar. They were pressed onto each sampling site for 10 s and correctly sealed. The plates were transported to the laboratory in a cooler bag and aerobically incubated at 37C for 24 h for evidence of microbial growth (ISO 6579 2002).

Statistical Analysis

Data on microbiological counts was first transformed to log (base 10) before analysis using excel work sheet for easy comparison and presented as means \pm standard errors. The effects of abattoir, slaughter stages and sampling day on the microbial count were analyzed using the Generalized Linear Model Procedures of the Statistical Analysis System (SAS, 2003). Significant differences among group means were tested using Least Significant Differences (LSD) and the statistical significance level was set at $\alpha = 0.05$. Results for the microbiological counts were also compared with the European Microbiological standards for meat (EC 2005).

The following model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \sigma_k + (\alpha\beta\sigma)_{ijl} + e_{ijl}$$

Where Y_{ijkl} = observed response (depended variable) (microbial count)

μ = overall mean,

α_i = abattoir effect,

β_j = effect of slaughter stage,

σ_l = effect of sampling day,

$(\alpha\beta\sigma)_{ijkl}$ = effect of the interactions,

e_{ijkl} = random residual error.

RESULTS

Aerobic Colony Counts From Carcasses at Different Stages During Slaughter

Generally, the mean log for ACC was observed to decrease along the slaughter line, that is, from the dirty area to the clean area (Fig. 1). For the LTA, after skinning and evisceration the mean log was 3.7 log CFU/cm². Washing the carcasses resulted in a slight decrease of about 0.2 log CFU/cm² in ACC mean

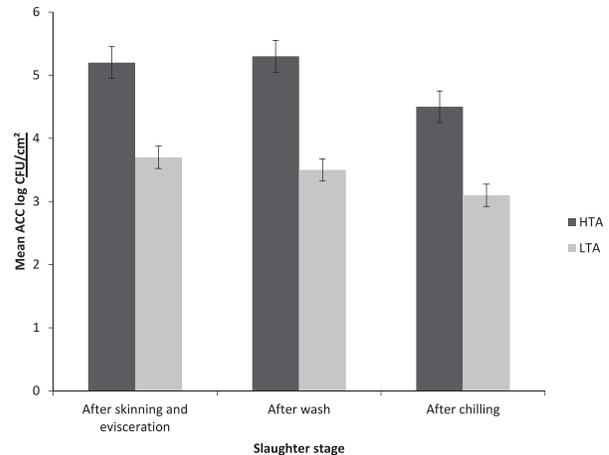


FIG. 1. MEAN LOG₁₀ OF AEROBIC COLONY COUNTS (ACC) AFTER SLAUGHTER, EVISCERATION, WASH AND CHILLING ON CARCASS SURFACES, AT $\alpha = 0.05$

log counts. Chilling reduced ACC on carcasses giving a mean log of 3.1 log CFU/cm² and this was significantly different ($P < 0.05$) with the mean log recorded after skinning and evisceration. On the other hand, the same trend was also observed in the HTA. Generally, mean logs were decreasing from skinning to chilling. However, washing with water resulted in a slight ACC mean log increase of about 0.1 CFU/cm² and chilling resulted in a further significant ($P < 0.05$) reduction. The mean logs at different stages along the slaughter lines in these two abattoirs differed significantly ($P < 0.05$); with HTA yielding comparatively higher mean scores (Fig. 1).

E. coli, *Salmonella* spp and *Enterobacteriaceae* From Carcasses at Different Stages Along the Slaughter Line

The mean log *E. coli* at different sampling stages in the LTA and HTA is presented in Fig. 2. After skinning and

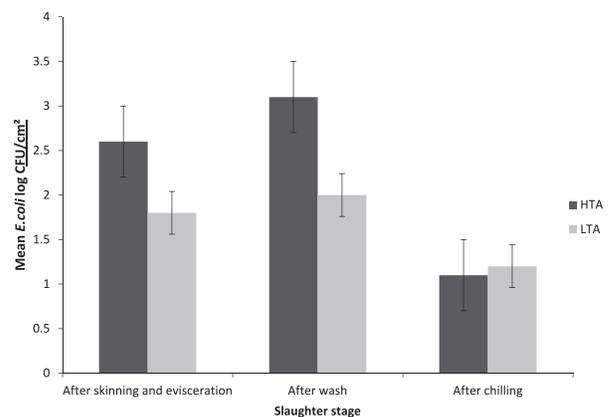


FIG. 2. MEAN LOG₁₀ OF *ESCHERICHIA COLI* COUNTS AFTER SLAUGHTER, EVISCERATION, WASH AND CHILLING ON CARCASS SURFACES, AT $\alpha = 0.05$

TABLE 2. LEAST SQUARE MEANS (LOG) ±STANDARD ERRORS OF ENTEROBACTERIACEAE COUNTS AFTER SLAUGHTER, EVISCERATION, WASH AND CHILLING ON CARCASS SURFACES

Abattoir	Number of carcasses	Number of swab samples	Slaughter Stage	Log CFU/cm ² (Mean ± SE)
High throughput abattoir	32	384	After skinning and evisceration	2.9 ^a ± 0.22
	32	384	After washing	3.6 ^a ± 0.22
	32	384	After chilling	0.2 ^b ± 0.31
Low throughput abattoir	20	240	After skinning and evisceration	1.5 ^c ± 0.26
	20	240	After washing	1.3 ^c ± 0.26
	20	240	After chilling	1.3 ^c ± 0.26

^{abc}Means in the same column with the same superscripts are not significantly different at $P < 0.05$.

evisceration the mean log was 3.4 log CFU/cm² in LTA and 5.1 log CFU/cm² in HTA. The HTA clearly yielded higher and significantly ($P < 0.05$) different mean scores than LTA. On the other hand, washing with water in LTA did not cause any significant ($P > 0.05$) changes in the mean log *E. coli*. However, chilling resulted in a significant ($P < 0.05$) reduction of about 0.8 log CFU/cm² in the mean log *E. coli*. At HTA, washing resulted in an increase in mean log *E. coli* by about 0.5 log CFU/cm² but not significantly ($P < 0.05$) different from the initial count. After chilling, significant ($P < 0.05$) changes in *E. coli* mean log were evident with a reduction of about 2.0 log CFU/cm². Overall, enumerated *E. coli* counts did not differ between the two abattoirs at these selected slaughter stages (Fig. 2). *Salmonella* was not detected at all sampled slaughter stages and carcasses.

Enterobacteriaceae mean log values for HTA and LTA at different stages during the slaughter process are presented in Table 2. All samples collected at each stage were positive and the mean log after skinning and evisceration ranged from 0.3 to 5.9 CFU/cm² with a mean of 2.9 log CFU/cm² in HTA. Significant differences ($P < 0.05$) were evident in HTA after chilling compared to after skinning and carcass wash, whereas for the LTA no significant ($P > 0.05$) differences were noted.

Mean Microbial Counts According to Day of Sampling and Water Analysis

Enterobacteriaceae, *E. coli* and ACC mean log counts according to days of sampling are presented in Table 3.

TABLE 3. LEAST SQUARE MEANS (LOG₁₀) AND ±STANDARD ERRORS OF AEROBIC PLATE COUNT (ACC), *E. COLI* AND ENTEROBACTERIACEAE COUNTS ENUMERATED AT DIFFERENT DAYS OF SAMPLING

Abattoir Type	Number of carcasses	Number of swab samples	Sampling day	ACC Log CFU/cm ² , (Mean ± SE)	<i>E. coli</i> Log CFU/cm ² , (Mean ± SE)	<i>Enterobacteriaceae</i> Log CFU/cm ² , (Mean ± SE)
Low throughput abattoir	30	384	1	3.5 ^a ± 0.41	2.0 ^a ± 0.19	0.9 ^a ± 0.21
	30	384	2	3.5 ^a ± 0.41	1.3 ^b ± 0.19	1.8 ^b ± 0.21
High throughput abattoir	48	240	3	5.1 ^b ± 0.09	2.5 ^a ± 0.17	2.7 ^c ± 0.20
	48	240	4	4.9 ^b ± 0.09	2.1 ^a ± 0.17	1.8 ^b ± 0.20

^{abc}Means in the same column with the same superscripts are not significantly different at $P < 0.05$.

The ACC log mean showed some significant ($P < 0.05$) difference between the two abattoirs. Although days 3 and 4 showed comparatively higher ACC counts than the first and second day of sampling. The *E. coli* mean log were not significantly different ($P > 0.05$) except for day 2 (1.3 log CFU/cm²) where the counts were observed to be lower. The mean log for *Enterobacteriaceae* was significantly different ($P < 0.05$) according to the day of sampling for all the abattoirs (Table 3).

Water samples from both abattoirs showed significantly ($P < 0.05$) higher microbial total viable count (TVC) regardless of the source of collection (Table 4). *E. coli* were only detected from the water samples from the HTA at the point of use. *Enterococci* were also detected in samples from the LTA at both the reservoir and point of use. However, no coliforms were detected in all water samples collected from both abattoirs. Generally, the number of micro-organisms increased from the reservoir to the point of use.

Bacteria Profile of Slaughtermen Hands and Equipment Used During the Slaughter Process

Evaluation of slaughtermen hands (Fig. 3) and equipment (Fig. 4) bacterial contamination showed that there is a general decrease in TVC from the dirty area to the clean area. For the equipment used in the LTA, bacterial load ranged from 10 to 4 CFU/cm², whereas in HTA it ranged from 7 to 3 CFU/cm² and the counts for hands ranged from 15 to 8 CFU/cm² in HTA and from 10 to 5 CFU/cm² in LTA.

TABLE 4. BACTERIAL PROFILE (CFU/100 ML) OF WATER FROM THE RESERVOIR TANK AND TAP SOURCES USED FOR CARCASS WASHING IN ABATTOIRS

Abattoir	Number of samples	Source	TVC CFU/mL, (35C, 48 h)	Total coliform			<i>Enterococci</i> MAC/100 mL (35C, 24 h)
				MAC/100 mL (35C, 24 h)	<i>E. coli</i> MAC/100 mL (35C, 24 h)	<i>E. coli</i> MAC/100 mL (44C, 24 h)	
Low throughput abattoir	6	Reservoir	3.0×10^2	0	0	0	1.0×10^0
	6	Tap	3.0×10^2	0	0	0	2.0×10^0
High throughput abattoir	6	Reservoir	2.5×10^1	0	0	0	0
	6	Tap	3.0×10^2	0	3.0×10^2	1.0×10^0	0

CFU= colony forming units.

Coliform = *E. coli* like bacteria.

MAC= maximum admissible concentration.

However, no *Enterobacteriaceae* were detected in all the workers' hands and equipment sampled. There were significant ($P < 0.05$) differences in TVC along the slaughter line and also between the abattoir.

DISCUSSION

According to Milios *et al.* (2014), for the proper identification and evaluation of abattoir hygiene weak points, slaughter process assessment based on microbial information is very important. From this study, it was evident that the equipment, slaughtermen hands and water can be sources of contamination during the slaughter process. This agrees with reports by Aslam *et al.* (2003), Nel *et al.* (2004), Bello *et al.* (2011) and Zweifel *et al.* (2014) who also identified the same weak points. According to McEvoy *et al.* (2004) and Zweifel *et al.* (2014), contaminants can be of fecal, soil, water or feed origin; they also reported that pre-slaughter environment and hides were also reservoirs of microbial contaminants. After skinning and evisceration, HTA yielded comparatively higher and significantly different ACC ($5.2 \log \text{CFU/cm}^2$), *E. coli* ($2.6 \log \text{CFU/cm}^2$) and *Enterobacteriaceae* ($2.9 \log \text{CFU/cm}^2$) carcass mean scores than LTA.

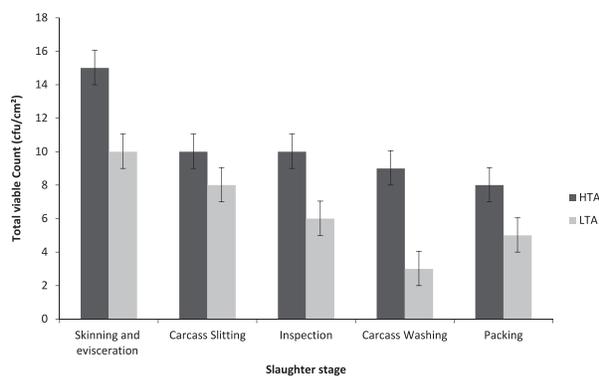


FIG. 3. MEAN LOG₁₀ OF TOTAL VIABLE COUNT (TVC) AT DIFFERENT STAGES DURING SLAUGHTER FOR SLAUGHTERMEN HANDS, ERROR BARS REPRESENT 95% CONFIDENCE INTERVALS

This is in contrast with previous reports by Bell (1997), Qiongzhen *et al.* (2004) and Zweifel *et al.* (2014) in which the counts did not exceed $2.0 \log \text{CFU/cm}^2$. However, they were still within the acceptable European Microbiological Standards (EC, 2005) and also in line with those reported by McEvoy (2004), Niyonzima *et al.* (2013) and Katsande and Govender (2014). Abattoir specific differences suggest that there were different levels of HMS implementation in these abattoirs during the slaughter process.

Washing with water in both abattoirs did not cause any significant changes in the mean log *E. coli*, ACC and *Enterobacteriaceae*; however, slight increases in counts were noted. This showed that the water did not effectively reduce microbial counts but rather resulted in the redistribution of the pathogens. This is in agreement with other reports by Bello (2011), McEvoy (2004), Loretz *et al.* (2011) and Zweifel *et al.* (2014) who noted an increase in bacterial load after washing the carcasses with water. Published information show that microbial contamination on skinned carcasses differ widely depending on processing environment, extent of hide-meat contact and

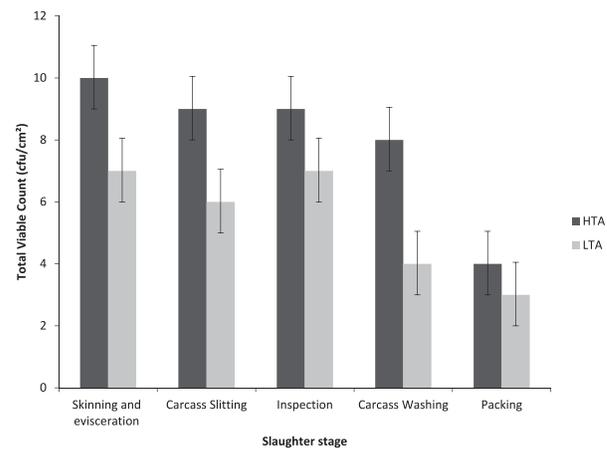


FIG. 4. MEAN LOG₁₀ OF TOTAL VIABLE COUNT (TVC) AT DIFFERENT STAGES DURING SLAUGHTER FOR SLAUGHTER EQUIPMENT, ERROR BARS REPRESENT 95% CONFIDENCE INTERVALS

water quality (Bello *et al.* 2011; Yang *et al.* 2012; Zweifel *et al.* 2014). From the current study, the water samples from both abattoirs showed significantly higher TVC and this could be the source of cattle carcass contamination during the slaughter process. World Health Organisation (2004) recommends the use of potable water for carcass washing with zero *E. coli* isolates. Use of low pressure hot water spray, high pressure cold water spray, steam pasteurization, acetic acid spray and irradiation have been reported to be very effective treatments to reduce carcass contamination during the slaughter process (Algino *et al.* 2007; Chen *et al.* 2012). From the assessed abattoirs there were no antimicrobial treatments applied and this could have attributed to the higher contamination levels observed.

Chilling significantly reduced ACC, *E. coli* and *Enterobacteriaceae* counts on carcasses, although HTA yielded comparatively higher scores. After chilling the ACC mean log from LTA was 3.1 and 4.5 log CFU/cm² for HTA and this is contradicted findings by Zweifel *et al.* (2005), Murray *et al.* (2001) and McEvoy *et al.* (2004) who found comparatively lower mean logs. According to Gill *et al.* (2003), Arthur *et al.* (2004), McEvoy *et al.* (2004) and Yang *et al.* (2012), chilling can result in an increase, decrease or no change to the microbial counts on carcasses depending on temperature, moisture, air speed, duration, carcass spacing as well as the sampled site. This could have been attributed to the varying microbial counts on the sampled carcasses and between abattoirs.

From the present study it was evident that the animals were exposed to different environmental conditions before and during slaughter. This is shown by the different microbial counts enumerated from the carcasses at different days of sampling. The mean log for *Enterobacteriaceae* differed significantly according to the day of collection, for both abattoirs. For *E. coli* the counts were only significantly different for the LTA. According to Callaway *et al.* (2003), LeJeune *et al.* (2004) and Buncic *et al.* (2014), these differences could be due to different animal management practices at the farm and abattoir hygiene. Though, Gill (2004) and Zweifel *et al.* (2014) have reported that microbial loads can vary significantly between animals as well as the sampled site. Washing animals before slaughter can help to reduce the variations in microbial load due to hides and lairages cleanliness. Chemical washing of animals before slaughter has been reported to significantly reduce *E. coli* and *Salmonella* counts on cattle carcasses (King *et al.* 2005). Veterinary inspectors can help in the inspection of animals to assess the level of cleanliness and to declare animals fit for human consumption based on the hygiene conditions.

Besides all the other factors discussed previously, slaughtermen and equipment can also be vehicles of pathogenic microorganisms. When hands and equipment are not

cleaned and disinfected properly, pathogens such as *E. coli* can be transmitted from one carcass to another or from personnel to carcass (Compos *et al.* 2009; Adzitey *et al.* 2011). In the present study, slaughtermen hands and equipment in the dirty area (skinning and evisceration) yielded more numbers of TVC compared to those in the clean area (carcass slitting, inspection, washing and packing) from both abattoirs. TVCs on hands were reported to be higher and this concurs with findings by Abd-Elalee *et al.* (2014). Our study findings are, however, in contrast with Boyce and Pitet (2002) who reported lower total bacterial counts which ranged from 3.9×10^4 to 4.6×10^6 CFU/hand. Moreover, Haileselassie *et al.* (2013) and Nel *et al.* (2004) also reported higher levels of carcass microbiological contamination in abattoirs and butcheries due to poor personal hygiene. However, the counts are within the acceptable range which shows that the hands and equipments were being decontaminated effectively. Conversely, no *Enterobacteriaceae* were detected in all the workers hands and equipment sampled. Hot water, sanitizers and detergents have been reported to have a significant effect in reducing microbial counts on slaughter equipment and hands (Griffith 2000; Redmond and Griffith 2003; Nel *et al.* 2004; Abd-Elalee *et al.* 2014).

CONCLUSIONS

Microbiological analysis of carcasses at different selected slaughter stages identified certain abattoir specific differences. It was evident that the equipment, slaughtermen hands and water can be sources of contamination during the slaughter process. Mean log (ACC, *E. coli* and *Enterobacteriaceae*) after skinning and evisceration for both abattoirs were generally higher compared to published data. Washing with water in both abattoirs did not cause any significant changes in the mean log *E. coli*, ACC and *Enterobacteriaceae*, though slight increases in counts were noted. Therefore, water might have contributed to the increase in carcass microbial counts due to use of non-potable water. Chilling significantly reduced ACC, *E. coli* and *Enterobacteriaceae* counts on carcasses in both abattoirs. Minor differences noted were probably related to the different chilling methods used by these abattoirs. Generally, slaughtermen hands and equipment in the dirty area (skinning and evisceration) yielded more bacterial counts compared to those in the clean area (slitting, inspection, washing and packing) from both abattoirs. For all the sampled carcasses, equipment and slaughtermen hands, HTA yielded comparatively higher bacterial counts than the LTA. However, *Salmonella* was not detected at all sampled slaughter stages. Identification of abattoir specific slaughter carcass contamination is important for the implementation and measure of the effectiveness of HACCP-based systems.

ACKNOWLEDGMENTS

Authors wish to thank the National Research Foundation (NRF) Innovation Masters and Doctoral Scholarship for Non-South Africans, DST-NRF Center of Excellence in Food Security project number P801, Govan Mbeki Research and Development Centre and Department of Livestock and Pasture, University of Fort Hare, Department of Rural Development and Agrarian Reform and staff at Grahamstown Veterinary Laboratory for their co-operation and co-ordination in all the financial issues pertaining to this study. Special thanks also go to the abattoir staff and workers for their cooperation during data collection.

REFERENCES

- ABD-ELALEEM, W.M.K., BAKR, W.A. and HAZZAH, N.O. 2014. Assessment of the hygiene and the bacteriological quality of butchers hands in some abattoirs in Alexandria, Egypt. *Food Control* 41, 147–250.
- ADZITEY, F., TEYE, G.A., KUTAH, W.N. and ADDAY, S. 2011. Microbial quality of beef sold on selected markets in the Tamale Metropolis in the Northern Region of Ghana. *Livest. Res. Rural Dev.* 23, 5–2.
- AGRICULTURAL RESEARCH COUNCIL (ARC). 2010. *Standards Operating Procedure for DPP 9704. Enumeration of Escherichia coli*, Feed and food analysis Laboratory, ARC, Onderstepoort Institute, South Africa.
- ALGINO, R.J., INGHAM, S.C. and ZHU, J. 2007. Survey of antimicrobial effects of beef carcass intervention treatments in very small state-inspected slaughter plants. *J. Food Sci.* 72, 173–279.
- ARTHUR, T.M., BOSILEVAC, J.M., NOU, X., SHACKELFORD, S.D., WHEELER, T.L., KENT, M.P., JARONI, D., PAULING, B., ALLEN, D.M. and KOOHMARAIE, M. 2004. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at various steps in commercial beef processing plants. *J. Food Prot.* 67, 658–665.
- ASLAM, M., NATTRESS, F., GREER, G., YOST, C., GILL, C. and MCMULLEN, L. 2003. Origin of contamination and generic diversity of *Escherichia coli* in beef cattle. *Appl. Environ. Microbiol.* 69, 2794–2799.
- BELL, R.G. 1997. Distribution and sources of microbial contamination on beef carcasses. *J. Appl. Microbiol.* 82, 292–300.
- BELLO, M., LAWAN, M.K., KWAGA, J.K.P. and RAJI, M.A. 2011. Assessment of carcass contamination with *E. coli* O157 before and after washing with water at abattoirs in Nigeria. *Int. J. Food Microbiol.* 150, 184–286.
- BOYCE, J.M. and PITTET, D. 2002. Guideline for hand hygiene in health-care settings: Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. *Infect. Control. Hosp. Epidemiol.* 23, 3–20.
- BUNCIC, S. 2006. Integrated food safety and veterinary public health. Wallingford, UK: CABI Publishing. *Int. J. Food Microbiol.* 146, 170–275.
- BUNCIC, S., NYCHAS, G.J., LEE, M.R.F., KOUTSOUMANIS, K., HEBRAUD, M., DESVAUX, M., CHORIANOPOULOS, N., BOLTON, D., BLAGOJEVIC, B. and ANTIC, D. 2014. Microbial pathogen controlling beef chain: Recent research advances. *Food Microbiol.* 32, 1–29.
- CALLAWAY, T.R., ANDERSON, R.C., EDRINGTON, T.S., GENOVESE, K.J., BISCHOFF, K.M., POOLE, T.L., JUNG, Y.S., HARVEY, R.B., and NISBET, D.J. 2003. What are we doing about *Escherichia coli* O157:H7 in cattle? *J. Anim. Sci.* 82, 93–99.
- COMPOS, A.K.C., CARDONHA, Â.M.S., PINHEIRO, L.B.G., FERREIRA, N.R., AZEVEDO, P.R.M., and STAMFORD, T.L.M. 2009. Assessment of personal hygiene and practices of food handlers in municipal public schools of Natal, Brazil. *Food Control* 20, 807–210.
- CHEN, J.H., REN, Y., SEOW, J., LIU, T., BANG, W.S. and YUK, H.G. 2012. Intervention technologies for ensuring microbiological safety of meat: Current and future trends. *Compr. Rev. Food Sci Food Saf.* 11, 119–232.
- COMMISSION REGULATION (EC) No 2073/2005. 2005. Microbiological criteria for foodstuffs, South Africa 26 p OJEU. 22.12.2005.
- DEPARTMENT OF AGRICULTURE FORESTRY AND FISHERIES (DAFF). 2000. Meat safety Act No. 40 of 2000. <http://www.nda.agric.za/daaDev/sideMenu/APIS/doc/MEATSAFETY.pdf>. (Accessed March 02, 2015).
- FOOD AND AGRICULTURE ORGANIZATION (FAO). 2004. Good practices for the meat industry, Animal production and health manual, Rome, Italy. <http://www.fao.org/docrep/007/y5454e/y5454e00.htm>. (Accessed April 02, 2015).
- GILL, C.O. 2004. Visible contamination on animals and carcasses and the microbiological condition of meat. *J. Food Prot.* 67, 413–419.
- GILL, C.O., BRYANT, J. and LANDERS, C. 2003. Identification of critical control points for control of microbiological contamination in processes leading to the production of ground beef at a packaging plant. *Food Microbiol.* 20, 641–650.
- GOVENDER, R., NAIDOO, D. and BUYS, E.M. 2013. Managing meat safety at South African Abattoirs. *Int. J. Biol. Food Vet. Agric. Eng.* 7, 124–229.
- GRIFFITH, C.J. 2000. Good hygiene practices for food handlers and consumers. In *Food-Borne Pathogens: Hazards, Risk and Control* (C.W. Blackburn and P.J. McClure, eds.) pp. 25–38, Woodhead Publishing Ltd, London.
- HAILESELASSIE, M., TADDELE, H., ADHANA, K. and KALAYOU, S. 2013. Food safety knowledge and practices of abattoir and butchery shops and the microbial profile of meat in Mekelle City, Ethiopia. *Asian Pac. J. Trop. Biomed.* 3, 407–412.
- HILL, A., BROUWER, A., DONALDSON, N., LAMBTON, S., BUNCIC, S. and GRIFFITHS, I. 2013. A risk and benefit assessment for visual-only meat inspection of indoor and outdoor pigs in the United Kingdom. *Food Control* 30, 255–264.
- HUDSON, W.R., MEAD, G.C. and HINTON, M.N. 1996. Relevance of abattoir hygiene assessment to microbial contamination of British beef carcasses. *Vet. Rec.* 139, 587–289.

- HEMMATINEZHAD, B., MESIPOUR, F., MOHAMMADI, M., DEHKORDI, F.S. and MASHAK, Z. 2015. Microbiological investigation of o-serogroups, virulence factors and antimicrobial resistance properties of shiga toxin-producing *Escherichia coli* isolated from ostrich, turkey and quail meats. *J. Food Saf.* 35, 491–500.
- INTERNATIONAL STANDARD ORGANISATION (ISO) 6579. 2002. *Microbiology of Food and Animal Feeding Stuffs – horizontal Method for the Detection of Salmonella spp.*, ISO, Geneva, Switzerland.
- INTERNATIONAL STANDARDS ORGANISATION (ISO) 17604. 2003. *Microbiology of Food and Animal Feeding Stuffs – Carcass Sampling for Microbiological Analysis*, ISO, Geneva, Switzerland.
- INTERNATIONAL STANDARD ORGANISATION (ISO) 4833. 2003. *Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Enumeration of Microorganisms-Colony-Count Technique at 30C*, ISO, Geneva, Switzerland.
- INTERNATIONAL STANDARDS ORGANISATION (ISO) 21528-2. 2004. *Microbiology of Food and Animal Feeding Stuffs-Horizontal Methods for the Detection and Enumeration of Enterobacteriaceae. II. Colony-Count Method*, Geneva, Switzerland.
- KATSANDE, T.C. and GOVENDER, R. 2014. A microbiological survey of fresh meat processed at abattoirs in Gauteng, South Africa. *J. New Gen. Sci.* 12, 29–22.
- KING, D.A., LUCIA, L.M., CASTILLO, A., ACUFE, G.R., HARRIS, K.B. and SAVELL, J.W. 2005. Evaluation of peroxyacetic acid as a post-chilling intervention for control of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on beef carcass surfaces. *Meat Sci.* 69, 401–407.
- LEJEUNE, J.T., BESSER, T.E., RICE, D.H., BERG, J.L., STILBORN, R.P. and HANCOCK, D.D. 2004. Longitudinal study of fecal shedding of *Escherichia Coli* O157:H7 in feedlot cattle: Predominance and persistence of specific clonal types despite massive cattle population turnover. *Appl. Environ. Microbiol.* 70, 377–384.
- LASOK, B. and TENHAGEN, B.A. 2013. From pig to pork: Methicillin-resistant *Staphylococcus aureus* in the pork production chain. *J. Food Prot.* 6, 1095–2108.
- LORETZ, M., STEPHAN, R. and ZWEIFEL, C. 2011. Antibacterial activity of decontamination treatments for cattle hides and beef carcasses. *Food Control* 22, 347–359.
- MCEVOY, J.M., SHERIDAN, J.J., BLAIR, I.S. and MCDOWELL, D.A. 2004. Microbial contamination on beef in relation to hygiene assessment based on criteria used in EU Decision 2001/471/EC. *Int J. Food Microbiol.* 92, 217–225.
- MILIOS, K., DROSINOS, E. H. and ZOIPOPOULOS, P. 2012. Factors influencing HACCP implementation in the food industry. *J. Hell Vet. Med. Soc.* 63, 283–290.
- MILIOS, K.T., DROSINOS, E.H. and ZOIPOPOULOS P. E. 2014. Food safety management system validation and verification in meat industry: Carcass sampling methods for microbiological hygiene criteria: A review. *Food Control* 43, 74–21.
- MURRAY, K.A., GILMOUR, A. and MADDEN, R.H. 2001. Microbiological quality of chilled beef carcasses in Northern Ireland: A baseline survey. *J. Food Prot.* 64, 498–502.
- NEL, S., LUES, J.F.R., BUYS, E.M. and VENTER, P. 2004. The personal and general hygiene practices in the deboning room of a high throughput red meat abattoir. *Food Control.* 15, 571–578.
- NIYONZIMA, E., BORA, D. and ONGOL, M. P. 2013. Assessment of beef meat microbial contamination during skinning, dressing, transportation and marketing at a commercial abattoir in Kigali city, Rwanda. *Pakistan J. Food Sci.* 23, 133–238.
- QIONGZHEN, L., JULIE, S.S. and CATHERINE, M.L. 2004. The presence of *Listeria*, *Salmonella*, *E. coli* and *E. coli* O157:H7 in Bison carcasses during processing. *Food Microbiol.* 21, 118–229.
- REDMOND, E.C. and GRIFFITH, C.J. 2003. Consumer food handling in the home: A review of consumer food safety studies. *J. Food Prot.* 66, 130–161.
- STATISTICAL ANALYSING SYSTEM (SAS). 2003. *SAS Users Guide: Statistics (Version 6th Ed.)*, SAS Institute Inc., Cary NC, Unites States of America.
- WORLD HEALTH ORGANISATION (WHO). 2004. *Guidelines for Drinking-Water Quality, 3rd Ed., Recommendations, Vol. 1*, WHO, Geneva. http://www.who.int/water_sanitation_health/dwq/GDWQ2004web.pdf. (Accessed 20 May 2014).
- YANG, X., BADONI, M., YOUSSEF, M.K. and GILL, C.O. 2012. Enhanced control of microbiological contamination of product at a large beef packing plant. *J. Food Prot.* 75, 144–149.
- ZWEIFEL, C., BALTZER, D. and STEPHAN, G. 2005. Microbiological contamination of cattle and pig carcasses at five abattoirs determined by swab sampling in accordance with EU Decision 2001/471/EC. *Meat Sci.* 69, 559–566.
- ZWEIFEL, C., CAPEK, M. and STEPHAN, R. 2014. Microbiological contamination of cattle carcasses at different stages of slaughter in two abattoirs. *Meat Sci.* 98, 198–202.