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Effects of Poor Post-slaughter Handling Practices on Microbiological Quality of Fresh Beef from Slaughterhouses in Kenya

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Abstract
Compliance to appropriate post-slaughter practices during meat processing is crucial for production of safe meat and meat products. Meat quality is highly influenced by the prevailing hygiene conditions during production and processing. Poor hygienic conditions can lead to meat contamination and spoilage by pathogenic bacteria hence food borne illnesses and subsequently meat losses. This study evaluated the effects of poor handling practices on the safety and quality of fresh beef from slaughterhouses mainly in pastoral regions in Kenya. About 95 meat and surface swab samples were collected and analysed for total viable counts, *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes*. The mean *Staphylococcus aureus* from the rump, neck, stomach and hind legs were 5.436, 6.153, 4.868 and 4.977 log $CFUg^{-1}$ respectively while that for *Listeria monocytogenes* was 2.259, 2.301, 2.301 and 2.392 log $CFUg^{-1}$ from rump, neck stomach and hind legs respectively. The highest mean of *Escherichia coli* counts (3.521 log $CFUg^{-1}$) was observed from the stomach. The mean TVC counts (6.339 log $CFUg^{-1}$) were observed at the neck region. The mean *S. aureus, E.coli* and TVC were 6.058, 4.504 and 4.491 log CFU/cm^2 from the weighing scale while mean *S.aureus*, *E. coli* and TVC from steel file were 6.161, 3.482 and 3.733 log CFU/cm^2 respectively. The means for *S.aureus, E.coli* and TVC from wedging knife were 5.926, 3.578 and 4.627 log CFU/cm^2 respectively. The means for *S. aureus*, *E. coli* and TVC were 6.141, 3.716 and 4.627 log CFU/cm^2 respectively from dust coats whereas those for *S. aureus*, *E. coli* and TVC were 6.264, 4.637 and 3.733 log CFU/cm^2 respectively from gumboots. The head cover also had its significant level of contamination with *S. aureus, E. coli* and TVC at 6.161, 3.690 and 4.491 log $CFUg^{-1}$ respectively. Means for *S. aureus* and *E. coli* from clothing, equipment and fresh meat were significantly different ($p<0.05$). The level of meat contamination was high and this was attributed to poor handling practices and hygiene conditions. This then necessitates the need for training and capacity building of slaughterhouses and personnel on the need for proper hygiene and good handling practices.
Keywords: Post-slaughter Practices; Microbiological Quality; Fresh Beef; Hygiene Practices

1. Introduction

There are many cases of food borne illnesses from consumption of contaminated meat which are reported every year in Kenya due to poor personal hygiene among meat handlers (Serraino et al., 2012, FDA, 2010). The main risk factor that leads to this contamination and subsequent food borne illnesses include poor personal hygiene, use of contaminated utensils/equipment and non-compliance with proper meat handling procedures (FDA, 2010, Huda et al., 2010). It has been reported that around 42% of food borne outbreaks in the USA are caused by poor hygiene and use of contaminated Personal Protective equipment (Aycicek *et al*., 2004). Personal hygiene encompasses general body cleanliness such as clean hands, short hair and short nails, clean personal protective equipment and personal health that are required to be free from communicable diseases (Francis and James, 2010).

Livestock production and meat processing and handling among the pastoral communities in Kenya is considered as the main source of protein and a source of income in most households (Huda et al., 2010). Meat is very nutritious and contains all the available nutrients for microorganisms to grow and survive (Huda et al., 2010). This increase the chances of food poisoning and mortality rate among the communities relaying on it as a source of protein (Nel *et al*., 2004; Yousuf *et al*., 2008). Muscle tissues of healthy living animal are free from microbes. However, meat contamination with pathogens occur during the process of slaughtering and handling external surfaces, water used in processing and intestinal contents (Al-Salamah, 2010). Equipment used in meat processing such as knives, saws, cleavers and hooks especially during the slaughtering operation makes a significant contribution to the microbial contamination of meat (Kivi et al., 2007). This is because they are in direct contact with hides and hair as well as exposure to contamination when in contact with steels, knife scabbards and the clothing of operator's (Grings, 2004).

The microbiological status of beef carcasses is highly dependent in most cases on the health status of the animals prior to slaughtering and processing as well as the hygienic condition of the slaughtering, processing and marketing environment (Kivi et al., 2007). It is therefore important to observe the hygiene practices to reduce meat contamination and subsequent food borne illness. This study evaluated the microbial contamination level of beef from poor handling and hygienic practices in selected slaughterhouses in pastoral regions of Kenya.

2 Methodology

2.1 Study design

A cross sectional study was used whereby 7 slaughter houses were pre-selected selected from the study regions namely Lodwar, Marsabit, Garissa, Kiserian, Kekonyokie, Bahati and Dagoreti. Meat samples were randomly collected from the rump, neck, stomach and the hind legs. Slaughter house equipment (wedging knives, steel file and weighing balance) were randomly selected and swabs taken for analysis. For PPEs the slaughter house operators were randomly selected and swabs on dust coats, caps and gumboots taken.

2.2 Study Region

The study was conducted in pastoral areas namely Marsabit, Turkana, Garissa, Kajiado, Nairobi and Kiambu.

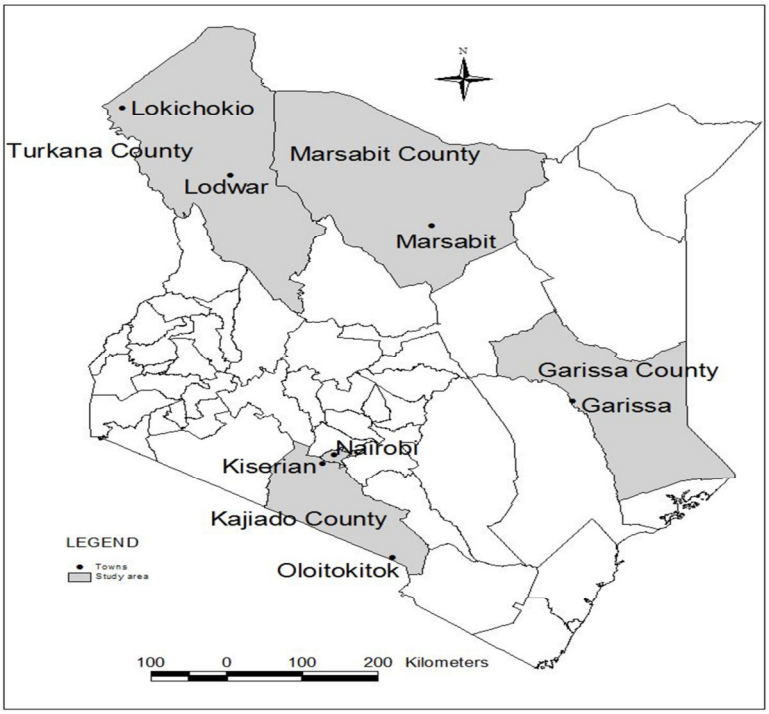

Figure 1: Map of Kenya showing the study areas

The counties were purposively chosen because they are the leading in livestock production. Nairobi (bahati and dagoreti) provided the basis for comparison between the pastoral practices and modern practices.

2.3 Sample collection and preparation

Meat and surface swab samples from equipment used for meat handling and Personal Protective Equipment for the workers in the slaughter house were aseptically collected for microbial analysis. Meat samples were collected from the following areas rump, neck, stomach and hind legs where 1gram pieces were cut from the areas transferred into screw-capped sterile labelled tubes containing 10 ml of buffered peptone water. For meat handling equipment (weighing scale and wedging knife) an area of 100cm^2 while for steel file an area of 10cm^2 was swabbed for 50 seconds with sterile moistened cotton wool swabs and the swabs transferred to the respective screw-capped sterile labelled tubes containing 10ml buffered peptone water. Personal hygiene samples were collected from gumboots, dust coats and caps by swabbing an area of 50cm^2 for 50 seconds with a sterile moistened cotton wool swab and transferred to labelled tubes containing 10ml of buffered peptone water.
Each test tube containing meat samples and surface swabs were thoroughly mixed using a vortex and opened aseptically by flaming the mouth part. 1ml of the sample was transferred to a small dilution bottle containing 9ml of buffered peptone water using sterile pipette tips. Serial dilution was carried out up to 10^{-6}.

2.4. Microbial analysis

Meat samples were analysed for *Staphylococcus aureus*, *Listeria monocytogenes*, total viable counts and *Escherichia coli* while swabs obtained from slaughter house equipment/utensils and personnel was analysed for *Escherichia coli*, *Staphylococcus aureus* and total viable counts using standard methods.

2.4.1 Determination of TVC

Total viable count was determined by pour plating 1ml of the 10-4, 10-5 and 10-6 sample dilutions using plate count agar. The plates were incubated at 350c for 48 hours and all grown colonies were counted and recorded

using colony counter.

2.4.2 Determination of *Staphylococcus aureus*

Colony counts of *Staphylococcus aureus* in meat were determined as described by ISO 6888-1 and ISO 6888-2. 28gms of Beird parker selective media was suspended in 1L of distilled water, the mixture was autoclaved at 121˚C for 15 minutes, allowed to cool to 45˚C and 50ml of egg yolk tellurite emulsion added. 1ml of each serial dilution of 10^{-4} to 10^{-6} was transferred to spread plates aseptically in duplicates and the plates incubated at 37˚C for 24hrs. Coagulase positive black colonies on the selective media were typical of *Staphylococcus aureus*.

2.4.3 Determination of E.coli

Enumeration of *E. coli* was done as described in ISO 16649-1, ISO 16649-2 and ISO 16649-3, 28.1g of *Brilliance* E. coli/coliform Selective media was suspended in 1 litre of distilled water. The media was gently boiled to completely dissolve, and then cooled to 45˚C; the molten media was then transferred to sterile plates. 1ml of each serial dilution of 10^{-4} to 10^{-6} was transferred to spread plates aseptically in duplicates and the plates incubated at 37˚C for 24hrs. A pink colony on the selective media was typical of *Escherichia coli*.

2.4. Determination of *Listeria monocytogenes*

1ml of 10^{-4}, 10^{-5} and 10^{-6} of sample dilutions were spread on listeria selective agar plates with added listeria selective supplement and incubated at 35^{0}c for 48 hours. Distinct listeria colonies were counted after incubation.

2.5 Statistical analysis

Microbial counts (CFU/g) were represented as log CFU/g. All the data was subjected to analysis of variance (ANOVA). The significance level was set at $p \leq 0.05$.

3 Results and discussion

3.1 Microbial counts (log CFU/g) from equipment used in slaughterhouses

The total viable counts in equipment used in slaughterhouses ranged from 4.627 to 5.359 log CFU/g with weighing scale recording the highest count of 5.359 log CFU/g while *S. aureus* was from 5.058 to 6.161with steel file recording the highest count of 6.161 log CFU/g for *E.coli* the counts ranged from 3.482 to 4.578 log CFU/g with weighing scale recording the highest count of 4.504 log CFU/g. Table 1 shows microbial counts from meat weighing scales, wedging knives and steel file in pastoral areas of Kenya. There was significance ($p \leq 0.05$) difference in levels of staphylococcus aureus on wedging knives and steel files used in the slaughter facility. This could be attributed to low levels of hygiene among the operators and not sanitizing the equipment.

Table 1: **Microbial counts (log CFU/cm^2) from equipment used in selected slaughter houses in pastoral areas of Kenya**

Selected equipment	N	Microorganisms*		
		S.aurues	***E.coli***	**TVC**
Wedging knife	10	5.926 ± 0.0231^{a}	3.578 ± 0.0537^{a}	4.627 ± 0.0119^{a}
Weighing scale	10	5.058 ± 0.0058^{a}	4.504 ± 0.0353^{b}	5.359 ± 0.3690^{b}
Steel file	10	6.161 ± 0.1019^{b}	3.482 ± 0.0217^{a}	4.823 ± 0.0387^{a}

*Values=means ± standard deviation, means in the same column with different superscripts are significantly different ($p \leq 0.05$). TVC=Total viable count and N=Number of samples

Discussion

There was a significant ($p \leq 0.05$) difference in levels of *Staphylococcus aureus* between the steel file and the wedging knife. There was no significant difference between the wedging knife and weighing scale. High levels of *S. aureus* indicated meat handling was poor in the areas. Higher levels of *S. aureus* indicated cross contamination from the clothing (gumboots) to the steel file as it was kept in the gumboots (Bredeeba *et al*., 2013). Continuous use of the steel file without washing or disinfecting might have accelerated the contamination further. Knives used have easy to clean surfaces but due to lack of sterilization equipment and infrequent sterilization, this leads to accumulation of microbes hence cross contamination (Bolton, 2006).

The mean TVC from wedging knives, weighing scales and steel files were 4.627, 5.359 and 4.823 log CFU/cm^2 respectively. High levels of TVC from the study are attributed to inadequate knife sterilization. Some of the slaughter operators kept their knives in the gumboots as they moved within the slaughter house. Some used a rag to wipe the knives and weighing scale this agrees with the finding of Chepkemoi *et al*.,(2015) who found out that butchery operators wiped the utensils with a piece of cloth. Results of the study were closely related to those of Ntanga *et al*., (2014) who found the mean values of knives and weighing scale to be 6.16 and 5.77 log CFU/cm^2 respectively. Chepkemoi *et al*., (2015) also found similar results from meat retail outlets in Nairobi. Higher levels of TVC are related to poor handling of meat handling equipment and inadequate cleaning and disinfection.

The levels of *E.coli* contamination were 3.578, 4.504 and 3.482 log CFU/cm^2 in wedging knives, weighing scale and steel file respectively. This could be attributed to use of contaminated water to wash or wipe the

equipment (Bhandare *et al*., 2009). Higher levels were recorded on the weighing scale because it was used frequently and the cloth used to wipe it was not washed regularly. It was also used to wipe the steel file which was kept in the gumboots instead of the scabbard (Ntanga et al., 2014) at this point cross contamination occurred.

3.2 Microbial counts (log CFU/g) from personnel clothes in slaughter houses

Microbial counts from personnel clothes in slaughter houses found in pastoral regions of Kenya are shown in Table 2. *S.aurues* levels ranged from 6.141 log CFU/cm^2 to 6.264 log CFU/cm^2 with gumboots recording the highest counts. This could be attributed to operators using the gumboots outside the slaughter facility and coming back to the facility without washing or disinfecting them. *E.coli* counts ranged from 3.690 log CFU/cm^2 to 4.637 log CFU/cm^2 with gumboots recording the highest count. There was no significant difference in the levels of *E.coli* in dust coats and caps. Total viable counts levels ranged from 3.733 log CFU/cm^2 to 4.627 log CFU/cm^2 with the highest counts being recorded on dust coats. There was a significant difference in the levels of total viable counts between dust coats and gumboots.

Table 2: Microbial counts (log CFU/cm^2) from personnel clothes in selected slaughter houses

Personnel clothing	N	Microorganisms		
		S.aurues	***E.coli***	**TVC**
Dust coats	15	6.141±0.0251^a	3.716±0.0084^a	4.627±0.0119^a
Cap	15	6.161±0.0881^a	3.690±0.0238^a	4.491±0.0140^a
Gumboots	15	6.264±0.0245^b	4.637±0.0152^b	3.733±0.0457^b

Values=means ± standard deviation, means in the same column with different superscripts are significantly different ($p \leq 0.05$). TVC=Total viable count and N=Number of samples

Discussion

Means for *Staphylococcus aureus* were 6.141, 6.161 and 6.264 log CFU/cm^2 respectively on dust coats, caps and gumboots. High levels of *Staphylococcus aureus* are attributed to not washing PPEs frequently as reported by Afnabi *et al.,* (2014). Low literacy level among slaughter house operators also contributed largely to high levels of contamination as they dint understand how dirty PPEs would influence final quality of meat (Chepkemoi *et al*., 2015). The study also agrees with findings of Ntanga (2013) who reported that not washing PPEs frequently would directly translate to meat contamination and subsequent spoilage.

The mean values for *Escherichia coli* were 3.716, 3.690 and 4.637 log CFU/cm^2 on dust coats, caps and gumboots respectively. High mean values were recorded on gumboots and this relates to the operators using the washrooms and not washing or disinfecting their gumboots. Osama *et al*., (2011) reported that slaughter house operators don't wash there PPEs or if they do wash then it's not adequate. Ali (2007) also found out that inadequate cleaning of PPEs would lead to meat contamination and subsequent losses. Lack of a water bath at the entrance of the facility to disinfect gumboots increase the challenge as the facility floor is loaded with microbes. Most of the flaying is done on the floor hence contamination (Serraino et al., 2012).

Mean for total viable counts were 4.627, 4.491 and 3.733 log CFU/cm^2 on dust coats, caps and gumboots respectively. Inadequate washing of PPEs or not washing at all could have been a major factor as reported by Chepkemoi *et al*., (2015). Not putting emphasis on personnel hygiene has contributed to food borne illnesses in meat producing areas (Kuria, 2005). Not cleaning PPEs leads to meat contamination hence food borne illnesses. Hygiene handling of meat is vital and emphasis needs to be put on the importance of good hygiene in meat production (Osama *et al*., 2011).

3.3 Microbial counts (log CFU/g) of meat samples from slaughterhouses in pastoral regions of Kenya

Table 3 show the microbial counts of meat samples from selected slaughterhouses in pastoral regions of Kenya sampled from different parts of the carcass; rump, neck, stomach and hind legs. The levels of *Staphylococcus aureus* ranged from 4.868 log CFU/g to 6.153 log CFU/g with the neck region recording the highest count. There was a significant difference in the levels of *Staphylococcus aureus* counts at the rump, neck, stomach and hind legs. The range for listeria was 2.259 log CFU/g to 2.392 log CFU/g with the highest count being recorded at the rump area. There was no significant difference in listeria counts at the rump, neck, hind legs and stomach. *E.coli* count ranged from 3.319 log CFU/g to 3.521 log CFU/g with the highest count being recorded at the stomach area. This could be attributed to spillage of the stomach contents during evisceration. There was no significant difference in *E.coli* counts at the four areas. Total viable counts ranged from 4.667 log CFU/g to 6.339 log CFU/g. The highest count was recorded at the rump area. There was a significant difference in the total viable counts at the four areas sampled.

Table 3: Microbial counts (log CFU/g) of meat samples from selected slaughterhouses

Meat sample	N	Microorganism			
		S.aurues	***l. monocytogenes***	***E.coli***	**TVC**
Rump	5	5.436±0.1799[a]	2.259±0.2413[a]	3.319±0.2024[a]	6.339±0.0721[a]
Neck	5	6.153±0.2023[b]	2.301±0.3010[a]	3.418±0.0208[a]	5.359±0.3690[b]
Stomach	5	4.868±0.1948[c]	2.301±0.0000[a]	3.521±0.0302[a]	4.667±0.0560[c]
Hind legs	5	4.977±0.0688[c]	2.392±0.3572[a]	3.329±0.2330[a]	4.823±0.0387[c]

*Values=means ± standard deviation, means in the same column with different superscripts are significantly different ($p \leq 0.05$). TVC=Total viable count and N=Number of samples

Discussion

The mean levels of *Staphylococcus aureus* were 5.436, 6.153, 4.868 and 4.977log CFU/g for rump, neck, stomach and hind legs respectively. Highest levels were recorded at the neck region while the lowest levels were recorded at the hind legs regions. High levels at the neck region are due to the meat coming into contact with the skin. After cutting the jugular crease some operators wipe the knife on the skin and use it a gain for flaying. This ultimately leads to contamination. *Staphylococcus aureus* is naturally present on the skin of animals as reported by Postgate, (2000). Some of the counts recorded in this study 5.436 and 6.153 log CFU/g were higher than the recommended 5.00 log CFU/g and are capable of producing enterotoxins to cause staphylococcal food poisoning (FDA, 2007). This has food safety concerns and something has to be done to reduce the contamination. Adzitey *et al*., 2011 also had the same findings as those in the table.

The *Listeria monocytogenes* for rump, neck, stomach and hind legs were 2.259, 2.301, 2.301 and 2.392 log CFU/g respectively. High levels of *Listeria monocytogenes* are attributed to cross contamination and recontamination from the meat handling equipment. Poor hygiene of the slaughter utensils might have greatly influenced the levels of listeria. Contamination of meat with listeria starts immediately after slaughter and is from dirty PPEs, hands and contaminated human and animal skin (Marinsek, 2002). Stephen, (2006) also found out that contamination from skin, equipment and PPEs leads to the spread of *Listeria monocytogenes.*

Mean levels of *Escherichia coli* for rump, neck, stomach and hind legs were 3.319, 3.148, 3.521 and 3.329 log CFU/g respectively. High levels of *Escherichia coli* are due to poor handling by the slaughter house operators and use of dirty personal protective equipment and flaying knives. Exposure of the meat to direct air also contributed greatly to high levels of *E.coli* and subsequent spoilage. Bhandare *et al*., (2007) reported poor hygiene in meat processing leads to meat contamination with *E.coli*. Soyiri *et al*., (2008) *E.coli* contamination is due to, meat coming into contact with faecal matter caused by poor handling of the stomach contents. This would then lead to food spoilage and subsequent food losses. Poor incision leads to leakage of intestinal contents onto carcasses which increase level of E.coli (Koutsoumanis et al., 2005). In the slaughter houses cold water is used to during the whole slaughtering process this water is ineffective in reducing microbial contamination than the recommended water at 74^0c hence increased microbial load (Zweifel et al., 2014). The water used might also act as a source of contamination.

The mean TVC from rump, neck, stomach and hind legs were 6.339, 5.359, 4.667 and 4.823 log CFU/g respectively. High levels of TVC in the study are attributed to poor handling practices leading to contamination of meat (FAO, 2007). The results of TVC obtained from the rump6.339 log CFU/g are higher than the recommended standard of less than 6.00 log CFU/g set by ICMSF (1985). The results of the study are higher than those of Ronoh *et al*., (2011) and Abdalla *et al*., (2010). Training on good hygiene practices and good production practices will help reduce on the level of contamination (Taoukis, 2005).

4. Conclusion

Meat from pastoral areas of Kenya was found to be contaminated with microbial pathogen including *E.coli, Listeria monocytogenes* and *S.aurues* which is attributed to poor handling practices post-slaughter and poor personnel hygiene. A well designed training programme and capacity building of abattoirs is crucial in addressing the current challenges in the meat trade of the interventions should include implementation of food safety management systems and HACCP including good hygiene practices (GHPs) and good handling practices (GHPs) in meat production. This will help to minimise of food borne illnesses and subsequent food waste and loss.

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