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**Microbiological analyses of beef slaughtering process and meat safety knowledge of
handlers at selected high and low throughput abattoirs**

By

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Declaration

I, Faith Nyamakwere, declare that this dissertation has not been submitted to any University and that it is my original work conducted under the supervision of Prof V. Muchenje and Dr B. Mushonga. All the citations and sources of information contained in this thesis have been duly credited.

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Prof Muchenje V.....Date

Dr Mushonga B.....Date

Abstract

Microbiological analyses of beef slaughtering process and meat safety knowledge of handlers at selected high and low throughput abattoirs

The objective of the study was to evaluate meat hygiene practices among meat handlers and microbiological analysis of beef carcasses, slaughtermen hands, equipment and water from low throughput (LTA) and high throughput (HTA) abattoirs in the Eastern Cape Province of South Africa. In stage one of the study, the level of knowledge, practices and attitudes towards meat safety and personal hygiene of slaughtermen were assessed. Forty workers directly involve in beef cattle slaughtering process were surveyed. Data were collected using a structured questionnaire, it contained questions on some of the most important meat safety cues such as knowledge, attitudes, personal hygiene and handling practices. Cross tabulation and Chi-square Test of Association were performed to examine the relationships between the demographic information and the knowledge regarding meat safety using the Statistical Package for Social Sciences (SPSS) version 22. Overall, a significant adherence to basic hygiene practices and a satisfactory level of knowledge were observed. Workers from a HTA yielded comparatively better statistically significant scores. Moreover, knowledge and practices of respondents were significantly associated ($P < 0.05$) due to educational level and professional training.

The second stage of the study involved evaluating the level to which cattle carcass, equipment, slaughtermen hands and water were contaminated with *Enterobacteriaceae*, *Salmonella*, *Escherichia coli* and aerobic colony counts (ACC) at different stages during the slaughter process (skinning, evisceration, carcass slitting, inspection, washing and packing). Cattle carcasses were sampled at four sites (rump, neck, flank and brisket) from a LTA (n= 240) and HTA (n= 384) abattoirs. Using conventional biochemical tests, HTA yielded significantly ($P < 0.05$) higher ACC (5.2 log CFU/cm²), *E. coli* (2.6 log CFU/cm²) and

Enterobacteriaceae (2.9 log CFU/cm²) carcass mean scores than LTA after skinning and evisceration. Specific abattoir hygiene differences were noted from washed and chilled carcasses. *Salmonella* was not detected across all sampled slaughter process stages. In addition, the equipment had bacterial load ranging from 10 to 4 CFU/cm² for LTA, whereas in HTA this was found to be 7 to 3 CFU/cm². The bacterial counts for slaughtermen hands were estimated to be 15 to 8 CFU/cm² in HTA and 10 to 5 CFU/cm² in LTA. Overall, 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 (P<0.05) bacterial counts than the LTA. Although the results showed a significant adherence to basic hygiene practices, some aspects such as routine medical examination, health certificates and professional training of slaughtermen still need to be improved. Therefore, these findings show that slaughtermen, equipment and water can be sources of contamination during the slaughter process.

Keywords: *Escherichia coli*, educational level, *Enterobacteriaceae*, health certificates, meat safety, medical examination, slaughter equipment, slaughter process

Dedication

This dissertation is dedicated to my family, particularly my mother Mrs S. Nyamakwere , my father Mr B. Nyamakwere, my siblings Josephine, Lindiwe, Trish and Bernard. Without their love and support this journey would not have been possible. Last but not least, my nephew Lambert, Blessed and my Niece Tessa, Kunashe and Atipaishe, your crazy jokes and smiles mean a lot to me. You will always be loved.

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List of Abbreviations

ACC- Aerobic Colony Count

CFU- Colony Forming Units

DAFF- Department of Agriculture, Forestry and Fisheries

DoH- Department of Health

EFSA- European Food Safety Authority

FSMS- Food Safety Management System

HACCP- Hazard Analysis and Critical Control Point

HAS- Hygiene Assessment System

HMS- Hygiene Management System

HTA- High Throughput Abattoir

LTA- Low Throughput Abattoir

NDA- National Department of Agriculture

SAMIC- South African Meat Industry Company

TVC- Total Viable Counts

VPN- Veterinary Procedural Notice

WHO- World Health Organisation

Chapter 1: General Introduction

1.1 Introduction

Meat consumption is increasing worldwide due to rapid population growth and urbanization (Fayemi and Muchenje, 2012). This has resulted in increased concerns and challenges of meat safety and hygiene (Sofos and Geornaras, 2010). The best strategy for improving meat safety is through implementation of appropriate hygiene schemes as well as educating and monitoring meat handlers (Sofos, 2008; 2009; Abd-Elaleem *et al.*, 2014). Therefore, meat safety regulations should be maintained from the slaughterhouse, processing, storage, distribution, retail outlets up until the products reach the consumers' table (Sofos and Geornaras, 2010). Government veterinary public health inspectors in South Africa oversee animal slaughter, processing and storage in abattoirs to verify compliance with meat safety Acts and regulations. The Acts include the Agricultural Products Standard Act, 1990 (Act No. 119 of 1990), Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972), Health Act No. 63 of 1977 and Meat Safety Act, 2000 (Act No. 40 of 2000) (National Department of Agriculture (NDA), 2004).

There are also various organizations in South Africa which ensure that the consumers are supplied with safe and wholesome meat. The South African Meat Industry Company (SAMIC) is one of the quality assurance organizations appointed by the National Department of Agriculture (SAMIC, 2013). It helps to enforce sections in the Agricultural Product Standard Act No. 119 of 1990 and in the Health Act No. 61 of 2003 (NDA, 2011) regarding meat safety practices. The Meat Safety Act No. 40 of 2000 and Red Meat Regulation No. 1077 of 2004 provide specific hygiene standards to be implemented during the slaughter process (DAFF, 2000; Government Gazette of South Africa, 2004). In addition, meat processing plants also implement good hygiene procedures based on the Hazard Analysis

Critical Control Points (HACCP) principles. This system has been regarded as an important measure of the effectiveness of Hygiene Management Systems (HMS) during processing (Govender and Genis, 2010). Therefore, in order to verify whether the HMS is working effectively, microbiological testing of meat is very important. Hygiene Assessment System (HAS) is used as an audit tool for the HMS in South African abattoirs. This is an acceptable method of ensuring optimal food safety since pathogenic microorganisms like *Escherichia coli*, *Salmonella enteritidis*, *Camplabacter jejuni*, *Listeria monocytogenes* and *Staphylococcus aureus* have been reported to be indicators of hygiene (Kramer *et al.*, 2000; Algino *et al.*, 2009; Lasok and Tenhagen, 2013). Therefore, visual assessment alone cannot be able to detect these pathogens.

In South Africa microbiological testing of meat is not compulsory; it is done mostly by abattoirs that supply bigger supermarkets. The Department of Agriculture, Forestry and Fisheries (DAFF) has established guidelines for microbial testing for export abattoirs as described in the Veterinary Procedural Notice VPN/IS/2010-01 (DAFF, 2000). Nevertheless, there is currently limited information on the microbiological quality of meat produced by these abattoirs on daily basis and how they can compare to the guidelines. The equipment, water and personnel involved during slaughter have also been reported to contribute to microbial contamination of the end product (Nel *et al.*, 2004; Bello *et al.*, 2011; Abd-Elaleem *et al.*, 2014). Therefore, slaughter process assessment is important for the proper identification and evaluation of abattoir hygiene weak points as well as possible sources of contamination.

1.2 Problem statement

Meat handlers have been reported to lack meat safety knowledge, adequate training and observed to be frequently engaged in poor handling practices, especially during the slaughter process (Nel *et al.*, 2004; Haileselassie *et al.*, 2013; Manguiat and Fang, 2013; Abd-Elaleem *et al.*, 2014; Jianu and Golet 2014). Gill and Baker (1998) and Yalcin *et al.* (2001) have shown that meat can be contaminated during the slaughter process. The first sources of contamination reported are the animals' skin and during evisceration, pluck removal and trimming (Gill *et al.*, 2003; Govender *et al.*, 2013). Therefore, care must be taken to prevent cross contamination. Reports also show that the redistribution of bacteria from the sites of initial deposition to other sites may be due to equipment (conveyor belts, saws, knife), contact surfaces, water and personnel (Nel *et al.*, 2004; Yang *et al.*, 2012; Arguello *et al.*, 2013). *Escherichia coli*, *Salmonella spp*, *Enterobacteriaceae* and fecal *streptococci* are some of the bacteria which have been identified as indicators of hygiene in abattoirs (Kramer, 2000; Algino *et al.*, 2009; Lasok and Tenhagen, 2013). These microorganisms appear mainly at the rumps, brisket, skin, hooves and shoulders of the carcasses (Wesley *et al.*, 2000). However, the current meat inspection criteria do not take these hazards into account. Visual assessment is mainly carried out.

1.3 Justification

There is need to assess the microbiological quality of meat and meat safety knowledge of meat handlers involved during the slaughter process. This will help identify any possible modes of contamination along the slaughter process and the responsible authorities can be able to take appropriate steps to improve safety (Abd-Elaleem *et al.*, 2014). Personal hygiene of meat handlers, proper sanitization of contact surfaces, utensils and use of clean water is important in order to prevent cross contamination or recontamination in abattoirs (Haileselassie *et al.*, 2013; Buncic *et al.*, 2014). Currently the Department of Agriculture, Forestry and Fisheries, Sub- Directorate Veterinary Public Health of South Africa, do not have a meat Microbiological Monitoring Programme (DAFF, 2010). Meat inspection is based mainly on visual assessment for cleanliness of which this cannot detect the most important food-borne hazards like *Escherichia coli*, *Salmonella* spp and *Campylobacter* (Hill *et al.*, 2013). Therefore, there is a need to develop more effective meat inspection programs in order to prevent contamination, food-borne illnesses and deaths. Such information can also help to come up with proper awareness campaigns, training sessions and monitoring strategies for meat handlers regarding environmental management, personal hygiene and food handling practices. According to published data, little information has been gathered regarding the microbiological quality of cattle carcasses, equipment and water used in abattoirs as well as meat safety knowledge and personal hygiene of slaughtermen in Eastern Cape Province abattoirs.

1.4 Objectives

The broad objective of the study was to determine the level of knowledge, attitudes and handling practices of the slaughtermen and also to determine the microbiological quality of cattle carcasses, slaughtermen hands, equipment, and water used during the slaughter process from two selected abattoirs in Eastern Cape Province.

Specific objectives

- a) To determine the level of knowledge and attitudes towards meat safety as well as personal hygiene and handling practices of slaughtermen from a low throughput abattoir (LTA) and high throughput abattoir (HTA)
- b) To determine the level of contamination with *Enterobacteriaceae*, *Escherichia coli*, *Salmonella spp* and aerobic colony count (ACC) of beef carcass at different slaughter process stages from a LTA and HTA. In addition the quantitative relationship between the carcass, slaughtermen hands, equipment and water microbiological quality was also determined.

1.5 Null Hypotheses

- a) All meat handlers involved in the slaughter process in selected abattoirs do not have adequate knowledge on meat safety, personal hygiene, attitudes and handling practices.
- b) The microbiological quality of beef, slaughtermen hands, equipment and water used during the slaughter process from two selected abattoirs, is the same.

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Chapter 2: Literature Review

2.1 Introduction

Raw meat and other meat products can be vehicles of hazards to human health. There are various types of hazards, which may be chemical, biological or physical. Biological hazards are of concern because the microorganisms or pathogens are found naturally in the environment or even on live animals (Sofos, 2014). Therefore, the occurrence of pathogens on raw meat can be due to different factors chief of which being; poor on farm animal management, improper slaughter practices, processing, storage conditions and lack of meat safety knowledge among meat handlers (Marais *et al.*, 2007). The consumer needs to be provided with wholesome meat and protected from consuming contaminated meat. This can be achieved by practicing good farm animal management, personal hygiene and providing all the meat handlers in the production chain with adequate knowledge on meat safety (Haileselassie *et al.*, 2013; Sofos, 2014). There is also a need for strict adherence and observation of rules and regulations set by the government in terms of standards and acceptable levels of pathogenic microorganisms by slaughter houses and retail outlets. Food premises should also apply good hygiene management systems. These systems help to identify, evaluate and control food safety hazards in order to achieve food safety requirements (NDA, 2011). The objective of this chapter is to review meat safety issues, acts, regulations and possible modes of contamination of raw meat from pre-harvest, slaughter, processing up to the retailer level.

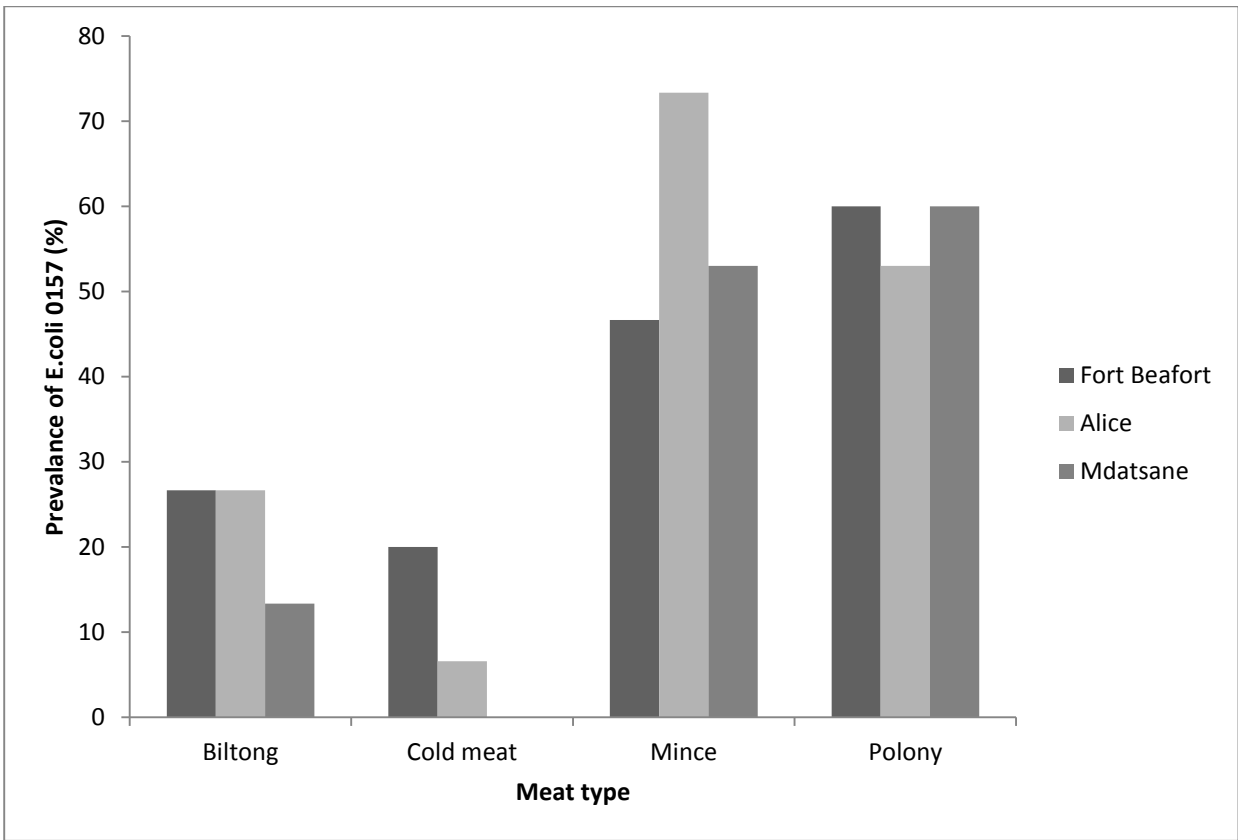
2.1 Overview of common food-borne pathogens in raw and ready-to-eat meat

Food-borne pathogens are disease causing microorganisms and these include bacteria, viruses, parasites, toxins and contaminants. Bacterial pathogens are more common and examples include *Escherichia coli*, *Salmonella spp*, *Listeria monocytogenes*, *Clostridium perfringes* and *Campylobacter spp* (Magwira *et al.*, 2005; Sofos, 2014). These are the most common pathogens of concern in fresh meat which causes illnesses and deaths in most developing countries. Viral pathogens such as Norovirus are also of a major concern at food service outlets and also considered as the major cause of food-borne diseases in the United States (Loader and Hobbs, 1999, Sofos, 2014). Common symptoms of food-borne illness include diarrhoea, abdominal cramps, vomiting and can result to long term effects like brain and nerve damage, kidney failure, chronic arthritis and finally death (Borgatta *et al.*, 2012). Anyone can suffer from food-borne diseases but groups such as pregnant women and older people with chronic illnesses are more susceptible (Soon *et al.*, 2013). These pathogens need to be carefully controlled since they can still be a problem in the future (Sofos, 2009). Control should start right from the farm up until the product reaches the consumer's table that is the farm to folk or farm to table approach (Sofos, 2010). Therefore, responsible authorities have to enforce control measures for these pathogens in order to improve meat hygiene and safety both locally and worldwide. Major pathogens that need to be controlled in fresh meat are briefly discussed in the following paragraphs.

2.1.1 *Escherichia coli*

Meat and meat products have been reported to be associated with disease outbreaks caused by different strains of *E. coli* (Magwira *et al.*, 2005; Kagambega *et al.*, 2012; Sallam *et al.*, 2013). *Escherichia coli* is a gram negative, rod shaped and facultative anaerobic bacteria which can be classified into different patho-groups according to their virulence traits. The common patho-groups are enteroaggregative *E. coli*, enterotoxigenic *E. coli*, Shiga toxin producing *E. coli*, enteroinvasive *E. coli*, enterohaemorrhagic *E. coli* and enteropathogenic *E. coli* (Nataro and Kaper, 1998; Mohammed, 2012). These pathogens can be found in the intestines of humans and domestic animals like sheep, goat and cattle (Momtaz *et al.*, 2013). Therefore, care must be taken during animal slaughter and processing to reduce chances of cross contamination. However, transmission to humans' is through consumption of contaminated meat which is undercooked and other meat products (Sallam *et al.*, 2013).

Previous studies carried out in countries such as Botswana, France, United Kingdom, Switzerland, Argentina found higher levels of *E.coli* in meat and meat products (Fanteli and Stephan, 2001; Vernozy-Rozand *et al.*, 2002; Magwira *et al.*, 2005). Current studies in South Africa also concur with these studies (Vorster *et al.*, 1994; Abong'o *et al.*, 2009; Ateba and Mbewe, 2013). Figure 2.1 shows the prevalence of *E.coli* in meat and meat products purchased from different retail outlets in Amathole district, South Africa. From this study mince meat and polony were found to be the ones with higher levels of *E.coli* from the three sampled towns (Abong'o *et al.*, 2009). The actual cause of contamination was not identified, which is why it is very important to assess the microbiological quality of the products at different stages during processing. This will help to come up with the actual cause of contamination and give room for proper improvement.



Adopted from: Abong'o *et al.* (2009)

Figure 2.1: Percentage prevalence of *Escherichia coli* isolated from meat and meat products purchased from different retail outlets in the Amathole district, Eastern Cape Province, South Africa

Kagambega *et al.* (2012) and Aluko *et al.* (2014) also demonstrated that raw meat in open markets is commonly contaminated by *E. coli*. This is as a result of poor personal hygiene of meat handlers and lack of meat safety knowledge. Therefore, meat handlers along the production, processing and distribution chain should be educated, carefully monitored and provided with enough equipment in order to prevent cross contamination. Consumers should also be educated on the appropriate meat handling, storage and heating practices.

2.1.2 *Listeria monocytogenes*

Listeria monocytogenes have been isolated from different dirty environments, including animal feed and waste, decaying garbage, stagnant water and sewages (Gomez *et al.*, 2014; Henriques *et al.*, 2014). This makes abattoirs and other retail outlets operating under unfavorable environments more vulnerable to this pathogen. *Listeria monocytogenes* are a problem in a variety of raw and ready-to-eat meat products and have the possibility to contaminate products after processing. The pathogen is able to survive and reproduce rapidly under diverse environmental conditions including cold below 3°C storage temperatures (Henriques *et al.*, 2014; Valero *et al.*, 2014). Therefore, proper hygienic practices should be applied during slaughter, processing, storage, transportation and marketing in order to destroy food-borne pathogens (Sallam *et al.*, 2013). *Listeria monocytogenes* can cause several types of diseases in humans including diarrhoea, abdominal cramps and listeriosis (Khanjari *et al.*, 2013). It is responsible for opportunistic infections, affecting people with weak immune system, pregnant women, new born babies and older people (Drevets and Bronze, 2008).

2.1.3 *Salmonella*

The salmonella genus comprises of more than 2500 different serovars, with *S. enteritidis* and *S. typhimurium* being the most common serovars causing illnesses in humans (Maka *et al.*, 2014). *Salmonella spp* are a major cause of salmonellosis infections in European countries, Poland, North America and most African countries (Zhao *et al.*, 2008; Maka *et al.*, 2014). Poultry products are the main sources of *Salmonella* infections (Oliveira *et al.*, 2005; Nierop *et al.*, 2005) and can be found in other foodstuffs such as beef, pork, dairy products, fruits and vegetables (Kubheka *et al.*, 2001; Velero *et al.*, 2014). *Salmonella* can contaminate the contents of an intact egg shell. Poor storage conditions, undercooking and cross-contamination by food handlers during processing help to promote spread and growth of the pathogen (Gornes-Neves *et al.*, 2014).

2.1.4 *Campylobacter spp*

Campylobacter spp are a cause of food-borne illness in most countries and the most common species responsible are *Campylobacter coli* and *Campylobacter jejuni* (Nobile *et al.*, 2013). They are found in the gastrointestinal tract of most wild and domestic animals with cattle, sheep, chicken, pigs and rodents being the main reservoirs (Kramer *et al.*, 2000; Padungtod and Kaneene, 2005). Infections caused by *Campylobacter spp* are associated with improper food preparation and consumption of undercooked poultry products, unpasteurised milk and unchlorinated water (Butzler, 2004; Kurincic *et al.*, 2005). That is why it is also very important to use potable water to wash carcasses in slaughter houses. Several studies have been conducted in different countries including Belgium (Ghafir *et al.*, 2008), Switzerland (Ledergerber *et al.*, 2003) on retail poultry products contamination with *Campylobacter spp*. These studies have also shown a decrease in the level of contamination of poultry products

for the past years from the farm to the market level. This suggests that processing plants are now implementing proper hygiene practices.

Table 2.1 shows the trend in prevalence of *Campylobacter spp* in poultry products from different parts of the world for the past few years. The table shows that for the sampled poultry products the prevalence of *Campylobacter spp* is decreasing. This can be attributed to the fact that that people involved in the production systems and consumers are now aware of meat safety issues. All these pathogens discussed are very important in the meat industry as they give guidelines or suggestive levels of pathogen contamination. Therefore, proper hygiene practices must be implemented right from the farm, during processing or slaughter up until the product reaches the consumers table. This will help to reduce chances or contamination with pathogenic microorganisms. Compliance with these hygiene regulations can be assessed by collecting samples at different processing stages for microbiological analysis. This is very important since the contamination of the final end product correspond to the combined contribution of different factors and stages along the meat processing chain (Niyonzima *et al.*, 2013; Zweifel *et al.*, 2014).

2.2 Guidelines for assessing the microbiological safety of raw and ready-to-eat meat on the market

Bacterial pathogens discussed above are referred to as indicator microorganisms and they give guidelines or suggestive levels of possible prevalence of pathogens on a given sample. These pathogens show the general hygiene and possible contamination levels (Brown *et al.*, 2000; Buncic, 2006). Microbiological pathogens that have been used as indicators in abattoirs and food outlets include *E. coli*, *Salmonella spp*, *Enterobacteriaceae*, *Listeria spp*, *Clostridium perfringens* and *Campylobacter spp* (Koochmaraie *et al.*, 2005; Algino *et al.*, 2009; Lasok and Tenhagen, 2013).

Table 2. 1: Prevalence of *Campylobacter* spp in poultry products

Study area	Prevalence Percentage (%)	Reference
North-Eastern Italy	81	Pezzotti <i>et al.</i> , 2003
South-Eastern Italy	73	Parisi, 2007
Central Italy	51	Sammarco, 2010
Catanzano Italy	45.7	Nobile <i>et al.</i> , 2013
Danish	0.3-1	Christensen <i>et al.</i> , 2013
Estonia	20.8	Maesaar <i>et al.</i> , 2014

However, there is no proven correlation between indicator organisms and prevalence or levels of pathogens per tested carcasses or meat portions. This is because microbiological examination is applied to random samples and does not cover the total surface area. Furthermore, each indicator is correlated to different pathogens therefore more than one indicator should be used (Brown *et al.*, 2000). The current legislation of European Union has set standards or acceptable levels for each of these indicator microorganisms and these are the ones applicable in South Africa at the moment (DAFF, 2010). Table 2.2 shows the microbiological standards and sampling plans for raw export meat of different animal species.

A three point class system is normally used for the classification of microbiological results for carcasses which are either satisfactory, acceptable or unsatisfactory (DAFF, 2010; European Food Safety Authority (EFSA), 2010). Satisfactory results indicate good microbiological quality and no meat safety action is required. Marginal results are borderline, that is, they are within the limits of acceptable microbiological quality but may indicate possible hygiene problems in the preparation of the food (Milios *et al.*, 2012). Unsatisfactory results are outside the acceptable microbiological limits and are indicative of poor hygiene or food handling practices. This may cause food-borne illness and immediate remedial action should be initiated (Milios *et al.*, 2014). There are slight differences in the microbiological levels accepted for raw and ready-to-eat meat. Table 2.3 shows the microbiological guidelines for different pathogens used as indicators of hygiene in ready-to-eat foods in retail outlets.

Table 2.2: Microbiological standards for European export meat

Category	Micro-organisms	Sampling plan		Limits	
		n ¹	c ²	m ³ (CFU/cm ²)	M ⁴ (CFU/cm ²)
Carcasses and meat cuts from cattle, sheep and goats	Aerobic colony count	35	7	3162 (3.5log)	100 000 (5.0 log)
	<i>E. coli</i>	35	7	1 (0 log)	10 (1.0 log)
	<i>Enterobacteriaceae</i>	35	7	31 (1.5 log)	316 (2.5 log)
	<i>Salmonella</i>	50	2	Absent/25g	Absent/25g
Carcasses and meat cuts from wild cloven hoofed game	Aerobic colony count	35	7	100 000 (5.0 log)	550 000 (5.7 log)
	<i>E. coli</i>	35	11	50 (1.7 log)	500 (2.7 log)
	<i>Enterobacteriaceae</i>	35	11	100 (2.0 log)	316 (2.5 log)
	<i>Salmonella</i>	50	2	Absent/25g	Absent/25g

Adopted from EC (2005)

¹n- the number of individual samples in a sampling plan

²c- the number of marginal samples allowed in 'n' samples

³m- defined value separating a good result from the marginally acceptable result

⁴M- the maximum value for a marginal result

Table 2.3: Guidelines levels for determining the microbiological quality of ready-to-eat food

Micro-organisms	S	M	US	P H
Enterobacteriaceae	$< 10^2$	10^2-10^4	$\geq 10^4$	-
<i>Escherichia coli</i>	< 3	3 – 100	≥ 100	Absent
<i>Clostridium perfringens</i>	$< 10^2$	10^2-10^3	10^3-10^4	$\geq 10^4$
<i>Bacillus cereus</i> and other pathogenic <i>Bacillus spp</i>	$< 10^2$	10^2-10^3	10^3-10^4	$\geq 10^4$
<i>Campylobacter spp</i>	not detected in 25g	-	-	Detected
<i>Salmonella spp</i>	not detected in 25g	-	-	Detected
<i>Listeria monocytogenes</i>	not detected in 25g	detected but $< 10^2$	-	$\geq 10^2$

Adopted from EC (2005)

Examination for the presence of pathogens in raw and ready-to-eat food products contributes to food safety. Therefore, the food industry has the duty to ensure that micro-organisms are eliminated or minimized to the extent that they cannot cause any harm to human health (DAFF, 2010). However, interpretation of results should be based on knowledge of the food product, production processes and care must be taken when interpreting results obtained in the absence of this information (Panisselo and Quantick, 2001). Slaughterhouses and retail outlets in South Africa have different quality control systems depending on production systems used.

2.3 Sampling and testing techniques of carcasses for hygiene evaluation in abattoirs

Sampling and testing of carcasses for hygiene criteria has been used by competent authorities during official inspection of slaughterhouses and also by the companies during Hazard Analysis Critical Control Point System (HACCP) validation and verification (Brown *et al.*, 2000; Milios *et al.*, 2014). This can be done by making use of different carcass sampling and testing techniques. Many researchers have used microbiological testing of bovine carcass surface in order to study the prevalence of different bacteria such as *Salmonella*, *E. coli* and *Campylobacter* during the slaughter process. In past years correlation between salmonellosis incidents in humans and consumption of contaminated food of animal origin had been established.

Meat contamination takes place in slaughterhouses during the slaughter process at different stages as reported by Adams (2014) and Arguello *et al.* (2013). Prevalence of different bacterial pathogens in animal faeces, hooves, skin, hides, and slaughter equipment, has been reported (Algino *et al.*, 2009). All this lead to meat contamination and the meat can be condemned or distributed to different retail outlets while it's contaminated. If proper hygiene practices are not exercised at this stage food-borne illness can occur. Therefore, proper

monitoring of abattoirs is very important and this can be done by responsible authorities making use of the set standards for inspection and evaluation.

2.3.1 Carcass sampling procedures for microbiological analysis in abattoirs

The microbiological testing for different indicators such as *Salmonella*, coliforms and *E. coli* can be performed at different sites of the carcass surface (Buncic *et al.*, 2014). Recommended sites include the ramp, brisket, thigh, flank and shoulders. Sampling should be performed at different stages during the slaughter process that is; after pelt removal, skinning, evisceration and pluck removal, washing, chilling and on the final product ready for redistribution to retailers (Ghafir *et al.*, 2008; Lasok and Tenhagen, 2013). In order to evaluate the effectiveness of a Food Safety Management System (FSMS) and obtain comparable and representative results microbiological data should be obtained from different stages and over a certain period. Sampling at the end of the process does not determine the contribution of each stage to the problem and therefore, cannot detect the cause of contamination (Luning *et al.*, 2011). Consequently, it is important to sample at each stage and also assess the equipment and personnel involved. This will help to identify the actual cause of contamination and give room for improvement. Microbiological results for carcasses immediately after chilling is always reported to be below, whereas after cutting or processing are high. This is due to the fact that the ratio of psychrotrophic to mesophilic bacteria is assumed to have been changed by the chilling process. However, other published data indicated that chilling can actually result in increase or no change of micro-organisms' numbers depending on the chilling method, air speed, temperature, duration, humidity, carcass site and spacing (Gill *et al.*, 2003; Arthur *et al.*, 2004; McEvoy *et al.*, 2004; Yang *et al.*, 2012).

Quality assurance systems like Hazard Analysis Critical Control Point System (HACCP) helps to ensure that meat produced from the abattoirs is of good quality and safe for human

consumption. However, the effect of HACCP involves many hygiene programs to properly evaluate its effectiveness. The programs or techniques should be capable of differentiating the effects of HACCP from other concurrent factors (Milios *et al.*, 2014). For the correct evaluation of hygiene practices in abattoirs microorganisms' referred to as indicators are used as discussed earlier. Koohmaraie *et al.* (2005), Ghafir *et al.* (2008) and Algino *et al.* (2009) showed a significant correlation between *E. coli* counts, *Enterobacteriaceae* counts and aerobic colony counts for cattle and pig carcasses. They concluded that *E. coli* counts and *Enterobacteriaceae* counts on carcasses were significantly higher in samples contaminated with *Salmonella*. Thus, according to the above authors, *E. coli* may be considered as a good indicator for enteric zoonotic agents such as *Salmonella* for beef and pork samples.

It has been reported that a large number of aerobic bacteria, such as *E. coli*, appear mainly at the rumps and the shoulders of the carcasses. The starting source of contamination was suspected to be dirty lairage areas and animals' skin. Other points of contamination include the hooves, evisceration, veterinary inspection and carcass trimming points. During evisceration, pluck removal and trimming, bacteria redistribute from the initial sites by the equipment used and personnel if proper hygiene practices are not followed (Luning *et al.*, 2011). Washing and chilling was found to decrease the *Salmonella* population of carcass significantly (Zweifel *et al.*, 2004). Therefore, care must be taken in order to reduce chances of contamination during the slaughter process and also encourage the use of potable water for washing.

2.4 Strategies for improving the safety of meat from farm to retail level

According to Buncic *et al.* (2014) and Sofos (2014) best strategies for improving meat safety include reducing contamination on live animals on farm, avoiding or minimizing cross contamination during slaughter, processing and at selling points. All these practices can be

achieved by applying a good farm animal management, educating the meat handlers and also proper monitoring by responsible authorities in all food premises (Haileselassie *et al.*, 2013). This helps to reduce chances of contamination and growth of surviving pathogens for meat intended for human consumption. If not monitored properly higher levels of contamination may result in food-borne diseases outbreaks. Therefore, food-borne pathogen control should start right from pre-harvest, post-harvest, processing, storage, distribution, retail outlets until it reaches the consumer's table.

2.4.1 Pre-harvest pathogen control

Since microorganisms are naturally found in the environment and in the animal's body, pathogen control measures should therefore start from farm level up until the meat reaches the consumers table. Pre-harvest pathogen control includes all measures and management practices at farm level to reduce the probability of having pathogens in animals and final meat products (Norrung and Buncic, 2008; Buncic *et al.*, 2014). This should aim to minimize sources, access, levels and transfer of contaminants to the animal. Technologies used to reduce pathogen levels in animals include diet manipulation (supplements), vaccines or immunization, effective bio-security and optimum animal welfare (Sofos, 2014). Proper animal management practices such as provision of clean water and feed, proper waste management in order to limit spreading of pathogens into the environment also help to reduce pathogen levels in animals (Buncic *et al.*, 2014). However, it might be difficult for farmers to control pathogens at this level due to lack of knowledge, resources and finance in some cases.

2.4.2 Quality and safety control systems in abattoirs

Quality and safety control in abattoirs should aim to minimize introduction of contaminants during slaughter, processing and distribution. This can be achieved by implementation of sanitization practices, proper personal hygiene of meat handlers and use of antimicrobial

interventions for inhibition of pathogen growth (Buncic *et al.*, 2014). Safety control in abattoirs is very important since it is a highly labour intensive working environment and many workers are involved, handling carcasses at different stages. Due to increased incidences of food-borne outbreaks around the world, many countries including South Africa have established quality inspection systems and regulations to be used in meat industries (NDA, 2004; Sofos, 2008). Quality control systems include application of the HACCP (Miliotis *et al.*, 2012; Miliotis *et al.*, 2014). Main prerequisites for the correct implementation of quality control systems and HACCP include commitment, financial support and they should be built on a solid foundation of pre-existing safety programs (Miliotis *et al.*, 2012).

The HACCP system is science based and systematically identifies, evaluate and control hazards that are significant for food safety. Food hazards can be microbiological, chemical or physical and these should be controlled throughout from processing to the end product. Reports from Zweifel *et al.* (2004) and Adams (2014) states that proper implementation of the HACCP systems should be based on measurable parameters like microbiological data gathered from different food industries during validation of the system. The microbiological results should be evaluated over a certain period and compared with standards set by the legislation (NDA, 2004; Buncic *et al.*, 2014). Microbiological parameters which have been used as indicators of poor hygiene in abattoirs as discussed earlier on include *Salmonella*, *Enterobacteriaceae*, *E. coli* and fecal *streptococci* and Total Viable Count (TVC) (Kramer, 2000; Algino *et al.*, 2009; Lasok and Tenhagen, 2013). However, most common meat pathogens associated with food-borne illnesses are *E. coli* O157:H7, non O157 STEC *E. coli*, *Staphylococcus aureus* and *Salmonella* (Koohmaraie *et al.*, 2005; Lasok and Tenhagen, 2013).

According to Miliotis *et al.* (2012), the implementation of HACCP in the food industry is difficult since this system is based on scientific facts and not human perceptions. Its success

requires inputs from different fields such as food engineering, food technology, microbiology and health (Milios, 2012). This system gained prominence because of increasing food-borne human illnesses reported worldwide. For the correct implementation of this system basic principles are involved which are; identification of hazards, establishment of critical limits, monitoring, establishment of corrective actions, verification and record keeping. However, the HACCP system does not stand alone since to some extent it will not be able to verify the cause of contamination (Buncic, 2006). Some of the programs which work hand in hand with HACCP include the Hygiene Management System (HMS), Good Manufacturing Programs (GMP), Hygiene Assessment Systems (HAS) and Quality Management Systems (QMS) (DAFF, 2010). These systems are considered as the backbone for better management and demonstration of meat safety in all meat processing plants in South Africa (Govender *et al.*, 2013). They are also implemented in accordance with sections in the Meat Safety Act No. 40 of 2000. Quality control systems for an abattoir should be well established, maintained, audited and documented. Poor management at abattoir level may alter all the efforts which would have been applied on farm. The control systems should be maintained even at retail level until the product reaches the consumers table.

2.4.3 Pathogen control at retail level

Personal hygiene of meat handlers, proper sanitization of contact surfaces and utensils is important in order to prevent cross contamination or re-contamination in abattoirs, butcheries, supermarkets and streets (Haileselassie *et al.*, 2013). Storage temperatures should also be controlled so as to inhibit multiplication, surviving and growth of existing pathogens. Research by Mosupye and Holy (2000), Kubheka *et al.* (2001), Manguiat and Fang (2013) and Abd-Elaleem *et al.* (2014) have shown that there is a food safety knowledge gap within butchery, supermarkets and abattoir meat handlers. A larger proportion of workers from

selected butcheries, shops and street vendors in Ethiopia were found operating without protective clothing, had no health certificates, hot water and sterilizer for cleaning the utensils and hands (Haileselassie *et al.*, 2013). Commonly isolated pathogens from such environments include *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* (Kagambega *et al.*, 2012; Haileselassie *et al.*, 2013; Aluko *et al.*, 2014).

Awareness programs and training is necessary for meat handlers to ensure food safety. However, if the meat is properly cooked it will pose little or no risk, regardless of poor sanitary environment and handling practices. The Government has set different acts and regulations to be implemented or followed by different processing plants (abattoirs) including retail outlets. This helps to ensure meat safety and reduces chances of food-borne illness.

2.5 Legislative control of meat safety in South Africa

Acts and regulations associated with safe, wholesome meat production give a clear guideline to producers and all those in the meat production chain on expected quality of meat and meat products by different government institutions involved (DAFF, 2012). Meat safety standards set by the government apply right from the farm up until the products reach the consumers table. These Acts and regulations provide basic key requirements for raw and ready to eat meat products. The control of meat safety in South Africa started way back in the 1960's and the Animal Slaughter, Meat and Animal Products Act No.87 of 1967 was in control (Govender *et al.*, 2013). The abattoirs were still owned and managed by the government. The government would employ health officers and inspectors to ensure safe animal slaughter, processing and distribution. Both ownership and meat inspection were later on privatized in the late 1980's but compliance is verified by state provincial veterinary services inspectors. That's when the Act No. 87 was repealed by the Abattoir Hygiene Act No. 121 of 1992. The act set out requirements for safe meat processing, abattoir hygiene and animal welfare. However, there was a decline in hygiene conditions in abattoirs and Act No. 121 was

repealed by the Meat Safety Act No.40 of 2000 (DAFF, 2000, Government Gazette of South Africa, 2004).

The Meat safety Act of 2000 promotes safety of meat and meat products, it also helps to establish and maintain abattoirs standards and all meat safety schemes in meat industry. The act made provision for the implementation of the HACCP based systems and different hygiene assessment schemes. These schemes include the Hygiene Management System (HMS), Good Manufacturing Programs (GMP), Hygiene Assessment Systems (HAS) and Quality Management Systems (QMS) (DAFF, 2010). They are regulated for all red meat abattoirs according to the Red Meat Regulation No. 1072 of 2004. The regulation contains some basic requirements for the personals involved in the meat value chain. Handlers should always wear protective clothing that is to cover their hair, hands and feet to prevent contamination (Government Gazette of South Africa, 2004). The clothes should be clean at all times, fingernails cut and no jewellery at all. Portable water should be available for sanitation purposes including hand washing facilities and sterilizers to be readily accessible for both the workers and visitors. The workers should also be effectively trained for issues regarding personal and environmental hygiene in food premises. Recent research by Manguiat and Fang (2013) and Abd-Elaleem *et al.* (2014) has shown that there is still a meat safety knowledge gap within food handlers in abattoirs and butcheries. Meat handlers should also have valid medical certificates all the times and go for regular tests for diseases like tuberculosis. They should not come to work or handle food if they are sick or suffering from any contagious diseases which can be transmitted via food. Haileselassie *et al.* (2013) found out that a larger proportion of meat handlers in abattoirs, butcheries and street vendors operated without valid certificates. Therefore, routine meat inspections should be performed by veterinarians, meat inspectors, animal health technicians or any other responsible authorities in order to assure safety (DAFF, 2010).

The Department of Forestry and Fisheries (DAFF) and Department of Health (DoH) mainly enforce safe meat production and processing (Govender *et al.*, 2013). Figure 2.2 shows how these departments interact and also other private entities involved during meat processing and distribution. The DAFF control farms and abattoirs as mandated by the Animal Diseases Act No. 35 of 1984 and the Meat Safety Act, Act No. 40 of 2000 made provision for the management and elimination of food animals with zoonotic diseases both at farm and abattoir level (Government Gazette, 2009). Once the products leave the abattoir premises supervision and control fall in the hands of the DoH. At the present moment there are also other Acts which work hand in hand with the Meat Safety Act, and these include the Agricultural Products Standard Act, 1990 (Act No. 119 of 1990), Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972) and Health Act No. 63 of 1977 (DAFF, 2010). This helps to ensure that meat supplied to consumers is of good quality, nutritious, wholesome, safe and culturally acceptable. However, meeting all these meat safety requirements remains a challenge due to financial constraints, ignorance of management authorities and low level of literacy of individuals involved in the production chain.

2.6 Summary of review

In conclusion, food-borne pathogen control should start right from farm (pre-harvest) to post-harvest, processing, storage, distribution, retail outlets until the product reaches consumers table. When assessing the safety of meat and meat products, it is very essential to rely on microbiological data. This is done by making use of indicator microorganism like *E. coli*, *Salmonella*, *Campylobacter spp*, *Listeria spp* and *Enterobacteriaceae* counts. These organisms give guidelines or suggestive levels of possible prevalence of pathogens on a given sample and general hygiene of the food premises. To limit possible chance of contamination in abattoirs; personal hygiene and meat safety knowledge of meat handlers is very important.

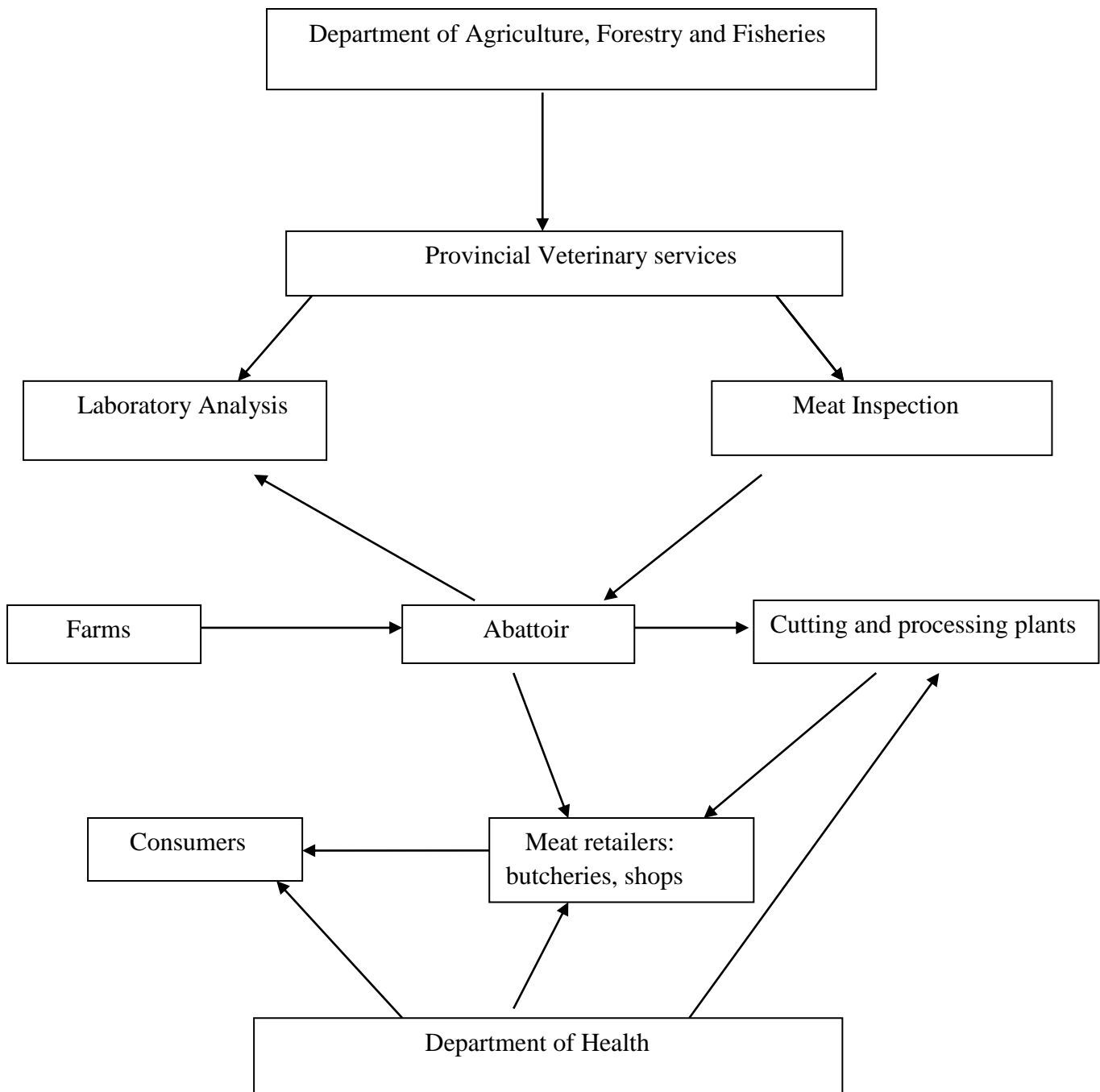


Figure 2.2: Regulatory role players in the meat industry of South Africa

This warrants further researches to validate the workers knowledge and general hygiene practices through microbiological correlation of meat samples. Local authorities should also inspect the premises, facilities and all activities to verify compliance with the standards and requirements according to meat safety Acts and Regulations.

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Chapter 3: Evaluation of meat safety knowledge, personal hygiene, attitudes and handling practices of slaughtermen from two selected abattoirs

Abstract

The objective of the study was to determine the level of knowledge, practices and attitudes towards meat safety and personal hygiene of slaughtermen from two abattoirs. A survey was conducted in which 40 workers from a low throughput (LTA: 15) and high throughput (HTA: 25) abattoirs were interviewed. Data was collected using a structured questionnaire comprising questions on slaughtermen knowledge and attitudes regarding meat safety as well as personal hygiene and handling practices. Cross tabulation and Chi-square Test of Association were performed to examine the relationships between the demographic information and the knowledge regarding meat safety. Results indicated that slaughtermen were generally ($P < 0.05$) adhering to proper ways of hand washing (LTA: 95.45%; HTA: 100%), drying (LTA: 46.67%; HTA: 90.91%) and wearing protective clothing during the slaughter process, with individuals from the HTA yielding comparatively better scores. The results further revealed that smoking around processing areas (LTA: 53.34%; HTA: 18.19%), cleaning and disinfecting working clothes, reporting illness (LTA: 66.66%; HTA: 37.28%), frequency of medical examinations, lack of health certificates (LTA: 26.67%; HTA: 40.91%) and professional training (LTA: 53.33%; HTA: 13.64%) were the main areas with knowledge deficiencies and practices among those interviewed. Knowledge and practices of respondents were significantly different ($P < 0.05$) according to educational level and professional training. Although the results showed a significant adherence to basic hygiene practices, some aspects such as routine medical examination, health certificates and professional training of slaughtermen still need to be improved.

Key words: educational level, health certificates, meat safety, medical examination, professional training, protective clothing

3.1 Introduction

The slaughter process is a highly labour intensive operation which involves many workers handling carcasses at different stages. Good slaughter hygiene practices have to be maintained during processing in order to reduce chances of microbiological contamination of the carcasses (Campos *et al.*, 2009; Haileselassie *et al.*, 2013; Zweifel *et al.*, 2014). According to Jumaa (2005), improper food handling and poor personal hygiene of workers contributes to approximately 97% of food-borne disease outbreaks amongst consumers and has led to deaths in some cases. Holt and Henson (2000) have also demonstrated a strong correlation between meat consumption and food-borne diseases outbreaks. Contamination of meat usually occurs during evisceration when gut contents come into contact with exposed meat. Other sources of contamination during the slaughter process can be the equipment, water, contact surfaces and personnel involved (Nel *et al.*, 2004; Govender *et al.*, 2013). Consequently, knowledge regarding meat safety and personal hygiene of slaughtermen is very important.

Reports from Pretoria, South Africa (Nel *et al.*, 2004), Western Romania (Jianu and Golet, 2014), Alexandria, Egypt (Abd-Elaleem *et al.*, 2014) have shown that most meat handlers lack meat safety knowledge, adequate training and are frequently engaged in poor handling practices. This result in cross-contamination of meat with pathogenic organisms like *Escherichia coli*, *Salmonella spp*, *Listeria monocytogenes*, *Clostridium perfringes* and *Campylobacter spp* (Bello, 2011; Zweifel *et al.*, 2014; Abd-Elaleem *et al.*, 2014). *Salmonella* and *E. coli* outbreaks in the United Kingdom (1980), United States (1993), Scotland (1993) Japan (1996) and Canada (1998) have raised consumer and regulatory concerns to improve food safety control measures (Armstrong *et al.*, 1996; Loader and Hobbs 1999; Hutter and Amodu, 2008). To prevent the spread of such pathogens, workers in food processing plants should be educated, trained, monitored and motivated to follow standard operational

procedures and regulations set by the responsible authorities (Jianu, and Golet, 2014). Meat handlers or workers play a major role in assuring that the meat supplied to the consumers is healthy and safe.

Currently, the food handling and processing plants are under increased consumer and regulatory pressure to improve the micro-biological safety of perishable raw commodities. In addition, the Meat Safety Act No. 40 of 2000 and the Red Meat Regulation No. 1077 of 2004 provides very specific hygiene standards regarding meat safety for the meat handlers to adhere to (DAFF, 2000; Government Gazette of South Africa, 2004). Therefore, the aim of this study was to assess the level of knowledge and attitudes towards meat safety as well as personal hygiene and handling practices of slaughtermen from two selected abattoirs in Eastern Cape Province, South Africa. It was also hypothesized that all slaughtermen do not have adequate knowledge and attitudes towards meat safety as well as personal hygiene and handling practices.

3.2 Materials and Methods

3.2.1 Study site

This study was carried out in two selected abattoirs which are in Grahamstown and East London from the Eastern Cape Province of South Africa. The abattoirs were classified into two major categories which were low throughput abattoir (Grahamstown: LTA) and high throughput abattoir (East London: HTA). Both abattoirs are approved by the competent authorities. The low throughput abattoir (LTA) slaughters approximately 18 animal units per day. All animal species use the same slaughter floor and only 15 workers are involved during the slaughter process. The HTA slaughters approximately 165 animal units per day. However, different slaughter floors are used for each animal species and approximately 25 workers were involved during the slaughter process.

3.2.2 Selection of respondents

A survey was conducted where a total of 40 slaughtermen were randomly selected from East London (HTA; 25) and Grahamstown (LTA; 15). The selection of respondents was limited mostly to those directly involved during the slaughter process. This includes those responsible for skinning, evisceration, meat inspection, washing and packing. Selection of these slaughtermen was done randomly.

3.2.2 Data collection

The study was approved by the University of Fort Hare Research Ethics Committee, with the certificate number MUS051SNYA01 (Appendix 2). Data were collected using structured questionnaires (Appendix 1) administered to randomly selected abattoir slaughtermen. The questionnaires were organised into different sections comprising of questions on slaughtermen demographic information, knowledge, attitudes, personal hygiene and handling practices regarding meat safety. The slaughtermen also answered questions pertaining to their personal health and actions taken when they are sick or injured at work. The respondents were interviewed with permission from the abattoir management staff during tea and lunch breaks. The participants were briefly informed about the purpose of the study before the interview and given a chance to ask for further clarity. They also signed an agreement as consent to participate freely without being forced; they were also assured of confidentiality for all the information provided (Appendix 1). The questionnaires were administered to the respondents by trained enumerators in a 10 minute one-on-one interview.

3.2.3 Statistical analysis

Descriptive statistics such as frequencies and proportions for the demographic information, meat safety knowledge and practices were computed using the Statistical Package for the Social Sciences (SPSS) version 22 (2000). Cross tabulation and Chi-square Test of

Association were performed to examine the relationships between the demographic information and the knowledge regarding meat safety, personal hygiene and handling practices. The level of statistical significance was set at $P < 0.05$.

3.3 Results

3.3.1 Socio-demographic characteristics of slaughtermen

The results of the socio-demographic information of slaughtermen interviewed in the two selected abattoirs in Eastern Cape Province of South Africa are shown in Table 3.1. The results revealed that the majority of the slaughtermen in both abattoirs, LTA (73.33%) and HTA (86.36%) were males. Most of the respondents from LTA (66.67%) were between the ages of 41 and 50 years, while 50.00% from HTA were between 21 and 30 years. In addition, 53.33% from LTA were married, while 54.55% from HTA were single. The majority of the slaughtermen from HTA (90.91%) had basic formal education, while only 53.34% from LTA had this level of education. Furthermore, a larger proportion of the abattoir workers (LTA: 93.33%; HTA: 95.45%) were observed to be from the Xhosa tribe.

3.3.2 Information regarding professional experience and health evaluation of slaughtermen

Information regarding the training and professional experience of the interviewed workers is shown in Table 3.2. The majority (73.33%) of the slaughtermen from LTA have been working in the meat industry for more than five years, while only 45.45% from HTA had this level of experience. A relatively larger proportion (86.36%) of workers from HTA had received professional training on meat safety and hygiene before being employed, compared to only 46.67% from LTA. Of those who attended the training from both abattoirs most had received the training only once (Table 3.2).

Table 3.1: Socio-demographic characteristics of meat handlers interviewed in two selected abattoirs in Eastern Cape Province South Africa

Characteristic	Category	*LTA		*HTA	
		Frequency	Percentage	Frequency	Percentage
Gender	Male	11	73.33	21	86.36
	Female	4	26.67	4	13.64
Age group	21-30	6	40.00	11	50.00
	31-40	4	26.67	7	27.27
	41-50	4	66.67	3	9.09
	≥50	1	6.67	4	13.64
Marital status	Single	6	40.00	13	54.55
	Married	8	53.33	10	40.91
	Divorced	1	6.67	2	4.55
Educational level	None	7	46.67	3	9.09
	Primary	7	46.67	4	18.18
	Secondary	1	6.67	16	68.18
	Tertiary	0	0.00	2	4.55
Tribe	Xhosa	14	93.33	23	95.45
	Zulu	1	6.67	2	4.55
Religion	Christianity	10	66.67	14	54.55
	Traditional	5	33.33	11	45.45

*LTA-Low throughput abattoir, *HTA- High throughput abattoir

Table 3.2: Information regarding training of meat handlers on meat safety and their professional experience

Characteristic	Category	*LTA		*HTA	
		Frequency	Percentage	Frequency	Percentage
Professional experience	<2 year	3	20.00	9	36.36
	2-5 years	1	6.67	5	18.18
	>5 years	11	73.33	11	45.45
Professional training	Yes	7	46.67	21	86.36
	No	8	53.33	4	13.64
Training sessions received	1	8	53.33	11	50.00
	2-5	0	0.00	3	9.09
	More than 5	0	0.00	7	27.27
	Never	7	46.67	4	13.64
Last training session(yr)	≤2	0	0.00	13	59.09
	2-5yr ago	1	6.67	2	4.55
	≥ 5 yr ago	7	46.67	6	22.73
	Never	7	46.67	4	13.64

*LTA-Low throughput abattoir; *HTA- High throughput abattoir

Furthermore, a relatively larger proportion from HTA (59.09%) attended the last session within the last two years, whereas 46.67% from LTA had not attended the training for more than five years.

Furthermore, the study revealed that most of the workers from LTA (73.33%) had valid medical certificates compared to only 59.09% from HTA (Table 3.3). However, the frequencies of going for routine medical examinations were comparable in both abattoirs, although LTA (60.00%) had higher scores than HTA (45.45%). On the other hand a smaller proportion (LTA: 20.00%; HTA; 4.55%) did not see the need to undergo medical examination. A relatively large proportion (91.9%) of the interviewed slaughtermen indicated that they have knowledge of food-borne diseases and have once been victims. The scores were not significantly different from both abattoirs. Vomiting (62.2%), diarrhea (51.4%), cholera (32.4%), abdominal pains (27.0%) and typhoid (2.7%) were ranked as the most common signs and symptoms of food poisoning. However, a relatively smaller proportion (13.5%) lacked knowledge of food-borne diseases. In this study, 13.5% indicated that they do not report to the management when they are sick, while 29.2% report sometimes. Of those who report a relatively larger proportion acknowledged that they are given medication and taken to the hospital by the management (Table 3.3).

Table 3.3: Practices for monitoring or maintaining the health status of meat handlers

Characteristic	Category	*LTA		*HTA	
		Frequency	Percentage	Frequency	Percentage
Valid Health Certificate	Yes	11	73.33	14	59.09
	No	4	26.67	11	40.91
Medical Examination	Every month	2	13.33	5	18.18
	After 6 months	1	6.67	8	31.82
	Annually	9	60.00	11	45.45
	No need	3	20.00	1	4.55
Report illness	Yes	5	33.33	17	72.73
	No	2	13.33	4	13.64
	Sometimes	8	53.33	4	13.64
Action/Treatment	Self-medication	3	20.00	6	22.73
	Traditional	5	33.33	4	13.64
	Pharmacy	2	13.33	1	4.55
	Hospital	5	33.33	14	59.09

*LTA-Low throughput abattoir; *HTA- High throughput abattoir

3.3.3 Personal hygiene and attitudes of slaughtermen regarding meat safety

All the respondents indicated that they always clean their hands before starting the slaughter process. In addition, approximately 89.2% of meat handlers from both abattoirs know the correct way of washing hands which includes the use of hand sanitizer, soap and hot water, while the remainder uses water only. A relatively larger proportion of the interviewed workers from HTA (90.91%) use disposable towels to dry hands compared to only 46.67% from LTA. On the other hand, others indicated that they use cloth towel, while 26.67% from LTA reported that they do not dry their hands. Above all, most slaughtermen (97.30%) from both abattoirs reported that they understand that washing hands helps to kill pathogens and to prevent contamination of the food.

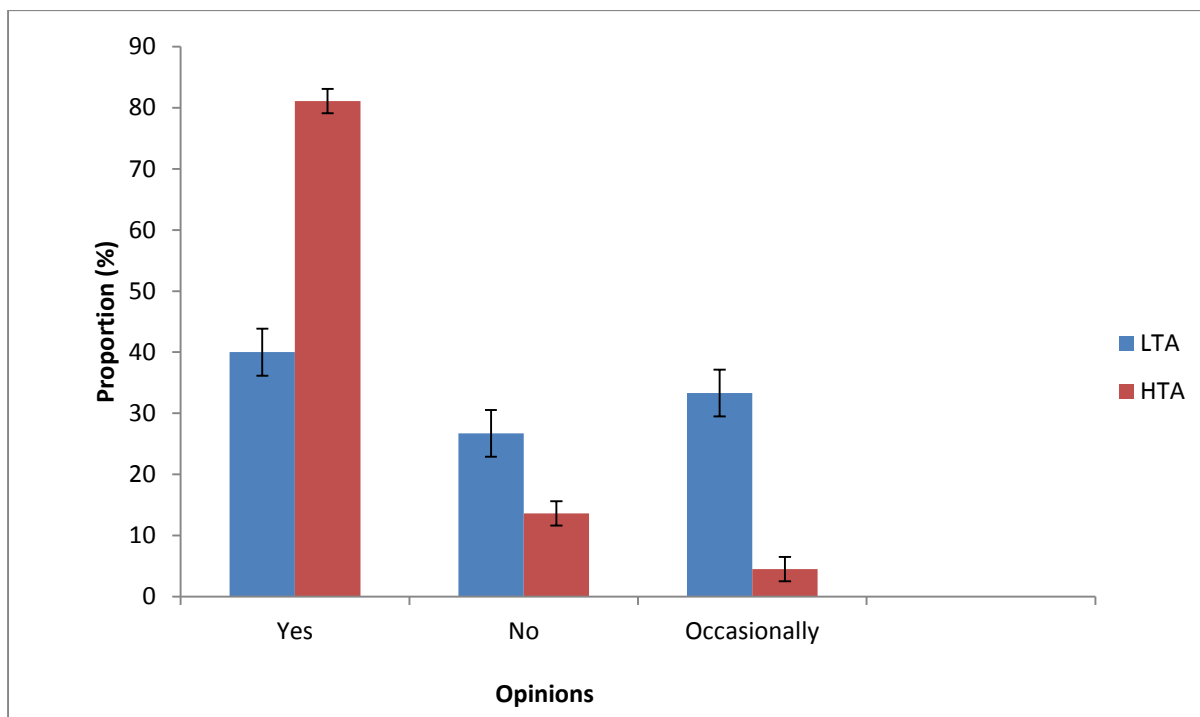
Without exception, all the slaughtermen from both abattoirs indicated that they wore aprons, hairnets, gumboots and overalls during the slaughter process. It was found that the majority of slaughtermen from LTA (53.33%) wore gloves sometimes, compared to the 59.09% from HTA who wore gloves always. The scores regarding this factor differed significantly ($P < 0.05$) between these two abattoirs. In addition, significantly higher number of slaughtermen ($P < 0.05$) with beards from HTA (77.27%) declared that they always wore beards-nets, while 9.09% wore sometimes and 13.64% never. On the other hand, a relatively larger proportion of slaughtermen from LTA (53.33%) reported that they sometimes wore beards-nets. Cleanliness of these protective clothing is very important. Results of the present study showed that the majority of slaughtermen from LTA (53.33%) wash protective clothing after three days, whereas majority from HTA (77.27%) wash daily. Furthermore, with regard to the frequency of disinfecting and cleaning of equipment such as knives and saws, HTA (95.45%) had the highest scores of those who indicated that they always cleaned compared to only 66.67% from LTA.

Smoking, eating and removing jewellery (rings, watches, and necklaces) are also considered as preventative measures which have to be implemented during the slaughter process. The majority of the respondents from HTA (81.80%) indicated that they remove jewellery before handling meat, compared to only 40.00% from LTA (Figure 3.1). The scores for both abattoirs differed significantly ($P < 0.05$). Furthermore, 46.67% and 81.82% of the respondents from LTA and HTA respectively, reported that they do not smoke during working hours or around processing areas (Figure 3.2). Scores for this factor differed significantly ($P < 0.05$) between the two abattoirs according to the Pearson Chi-square test which was performed.

3.3.4 Comparative analysis of slaughtermen knowledge and practices on meat safety

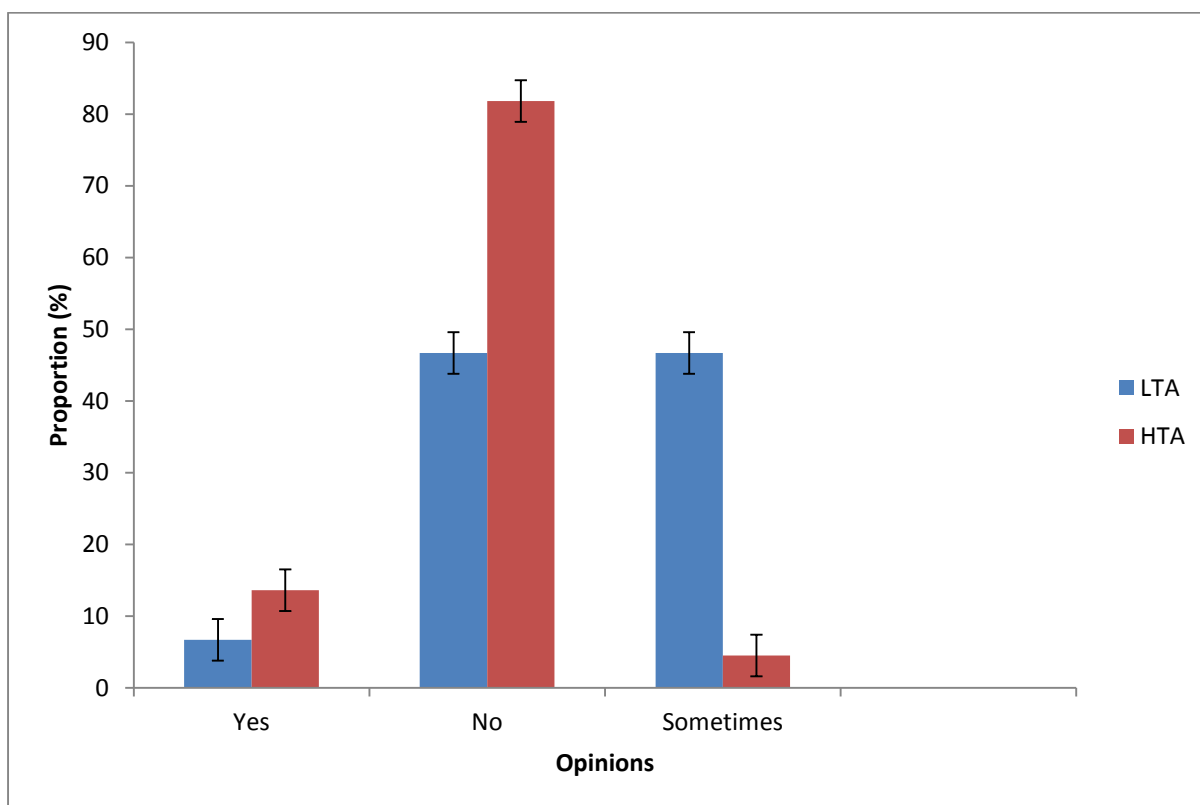
The demographic information of slaughtermen interviewed was observed to be associated with some of the personal hygiene practices, attitudes, beliefs and knowledge regarding meat safety. Level of knowledge of food-borne diseases and general handling practices were observed to be increasing with age, even though no statistical significance ($P > 0.05$) could be detected for most of them. There were no significant differences between males and females for most of the observed meat safety knowledge and practices. However, females were observed to clean and disinfect equipment ($P < 0.05$) and working surfaces ($P < 0.05$) more frequently compared to males.

The level of education was also seen to be associated with some of the respondents' knowledge and practices regarding meat safety. A monotonic increase in knowledge about food-borne diseases could be observed increasing along the education level. There were significant differences ($P < 0.05$) for most practices observed, that is, reporting illness, wearing gloves, frequency of cleaning and disinfecting equipment and working clothes (Table 3.4). The majority of respondents indicated that they always report any illness and the frequency of reporting could be seen increasing along the educational levels.



*LTA-Low throughput abattoir; *HTA- High throughput abattoir

Figure 3.1: Personal hygiene practice (removal of jewellery) before starting the slaughter process, error bars represent 95% confidence intervals.



*LTA-Low throughput abattoir; *HTA- High throughput abattoir

Figure 3.2: Personal hygiene practice (smoking around processing areas) before starting the slaughter process, error bars represent 95% confidence intervals.

Table 3.4: Associations between educational level and slaughtermen general personal hygiene and handling practices regarding meat safety

Hygiene practice	Educational level (proportion %)				Significance
	None	Primary	Secondary	Tertiary	
Reporting illness					
Yes	55.6	18.2	81.3	100	
No	0.0	36.4	6.3	0.0	*
Sometimes	44.4	45.5	6.3	0.0	
Frequency of disinfecting working clothes					
Daily	0.0	45.5	81.3	100	
After 2days	33.3	27.3	6.3	0.0	**
After 3days	66.7	27.3	12.5	0.0	
Frequency of disinfecting contact surfaces					
Always	44.4	45.5	100	100	**
Sometimes	55.6	54.5	0.0	0.0	
Frequency of wearing gloves					
Always	0.0	27.3	68.8	0.0	
Sometimes	55.6	45.5	18.8	100	*
Never	44.4	27.3	12.5	0.0	

***Significant at *P<0.05, **P<0.01

On the other hand, without exception, all the respondents know the importance of washing hands and wearing protective clothing regardless of their educational level. A monotonic increase in meat safety knowledge can be seen as the professional experience years increases. Respondents with three and more years of experience were observed to have better knowledge of food-borne diseases and other hygienic practices during the slaughter process. However, the scores do not differ significantly ($P > 0.05$) according to the Pearson Chi-square test, either for the knowledge or hygienic practices. Regarding professional training, there were significant differences for most practices between trained and untrained workers. With majority of the workers who received training yielding higher and better score regarding meat safety (Table 3.5).

Table 3.5: Associations between professional training and slaughtermen general personal hygiene and handling practices regarding meat safety

Hygiene practice	Professional training (proportion %)		Significance
	Yes	No	
Reporting illness			
Yes	69.2	27.3	
No	15.4	9.1	**
Sometimes	11.5	63.6	
Frequency of disinfecting equipment			
Always	96.2	54.5	**
Sometimes	3.8	45.5	
Frequency of disinfecting working clothes			
Daily	69.2	9.1	
After 2days	3.8	54.5	**
After 3days	26.9	36.4	
Frequency of disinfecting contact surfaces			
Always	80.8	45.5	*
Sometimes	19.2	54.5	
Frequency of wearing gloves			
Always	50.0	9.1	
Sometimes	34.6	45.5	*
Never	15.4	45.5	

***Significant at *P<0.05, **P<0.01

3.4 Discussion

This study has led to the identification of differences between knowledge and personal hygiene practices regarding meat safety of slaughtermen operating in two selected abattoirs in Eastern Cape Province. Different interesting patterns have been observed that will add new information or knowledge for improvements in implementation of standard operational procedures by the responsible authorities in the meat industry. Personal hygiene practices investigated in this study include washing of hands, smoking, eating, removal of jewellery (rings, watches, and necklaces), the wearing of protective clothing, cleaning and disinfection of working clothes and equipment. These practices are considered as mandatory preventative measures which have to be implemented during the slaughter process to reduce chances of cross contamination (Nel *et al.*, 2004).

According to the Red Meat Regulation No. 1077 of 2004 (section 49), hand-washing basins, sterilizers and disposable towels should be made accessible to both abattoir workers and visitors (Government Gazette, 2004). In this study, all respondents indicated that they always clean hands before starting the slaughter process which contradicts reports by Gomes-Neves *et al.* (2007), Soon and Baines (2012), Abd-Elaleem *et al.* (2014) and Jianu and Golet (2014). In addition, upon asking the workers what they use for hand washing, 89.2% from both abattoirs indicated that they use hand sanitizer, soap and hot water. These results are higher than findings by Abd-Elaleem *et al.* (2014) where only 37% indicated that they use hot water and soap. Shojaei *et al.* (2006) reported that correct hand hygiene results in a microbial count reduction from approximately 72.7% to 32%.

Hand drying is the final and an essential component of effective hand washing (Abd-Elaleem *et al.*, 2014). Damp hands can result in skin excoriation leading to a higher number of bacterial colonization and facilitate the spread of pathogens (Jamaa, 2005). Soft, absorbent paper towels are recommended for drying hands than the use of a cloth. Cloths have been

reported to be ineffective in removing microorganisms, thereby increasing the chance of cross contamination (Fawzi *et al.*, 2009). In the present study, workers from HTA (90.91%) are adhering to the proper practices. This can be attributed to the fact that interviewed meat handlers (97.30%) have the knowledge that washing hands helps to kill pathogens and to prevent contamination of the food. These findings are in contradiction to those reported by Abd-Elaleem *et al.* (2014), but agree with those reported by Nel *et al.* (2004).

Smoking or eating during work or wherever food is processed or handled is prohibited, since the fingers can come into contact with lips and saliva increasing chances of contamination. In the present study, a relatively large proportion of respondents from LTA (53.4%) indicated that they do smoke or occasionally smoke during breaks compared to only 18.1% from HTA. In contrast, earlier researches by Nel *et al.* (2004), Jianu and Golet (2014) and Abdul-Mutalib *et al.* (2012), have indicated that respondents reported that they neither smoke nor eat inside processing areas. Smoking may cause coughing thus, transferring aerosols containing microorganisms to the food (Gordon-Davis, 1998). Therefore these workers should be encouraged to properly wash their hands before resuming slaughter process activities. Routine medical examinations are mandatory for meat handlers in order to assess their general health.

Regulation No. 1077 of 2004 section 57 states that, before employment workers should produce a medical proof that they are healthy and not carriers or suffering from a communicable disease. The current study noted that 20% of workers from LTA do not know the importance of going for medical exams unlike in the HTA where only one respondent (4.55%) reported lack of this knowledge. This finding indicates a gap in meat handlers' knowledge about health and food contamination. On the other hand, most meat handlers from LTA (26.67%) and HTA (40.91%) indicated that they do not have valid health certificates. Similar to this study, Haileselassie *et al.* (2013) and Abd-Elaleem *et al.* (2014) also noted that upon inspection most workers did not have valid health certificates. Harker (2001)

highlighted that it is important to do a pre-employment health assessment for food handlers and inclusion of routine salmonellae screening sessions at least every year (Feglo *et al.*, 2004).

Meat handlers are also encouraged to report illnesses such as diarrhea, sore throat, fever, cold or open lesions to the supervisor or management so that appropriate measurements are taken. Reporting and taking a leave when sick is very important when working in the food premises to prevent or eliminate chances of cross contamination. A relatively large proportion of meat handlers from LTA (72.73%) indicated that they always report any illness to the supervisor, while only 53.33% from HTA declared that they report sometimes. These findings are lower than those recorded by Nel *et al.* (2004), where 96.4% of the respondents indicated that they report always. Nel *et al.* (2004) also reported that all the respondents declared that whenever they report an illness they were sent to the hospital, whereas in the present study only few respondents indicated that they are taken to the hospital when sick (LTA: 33.33%; HTA: 59.09%). The study has also revealed that most respondents have knowledge of food-borne diseases and have once been victims. According to Hansen *et al.* (2003), past experience with food-borne diseases results in higher levels of common concern and risk perceptions.

Wearing of protective clothing is one of the major measures implemented in the food industry. It helps to prevent cross contamination. Protective clothing helps to protect both the food product and the meat handler from cross contamination (Muinde and Kuria, 2005). Hairnets and beard-nets specifically help to prevent loose hairs and also dandruff from falling into the food since hair is reported to be a source of *Staphylococcus aureus* (Abd-Elaleem *et al.*, 2014.) It also helps to discourage workers from scratching their scalp and later on handling meat without properly washing hands. All respondents from the present study declared that they always wear aprons, hairnets, gumboots and overalls. Similar findings were also recorded by Nel *et al.* (2004) while in contrast; Haileselassie *et al.* (2013) and Abd-

Elaleem *et al.* (2014) observed that most of the meat handlers work without protective clothing. However, frequency of wearing beard-nets and gloves was significantly different between these two selected abattoirs. Therefore, the workers need to be properly trained and also provided with adequate protective clothing in order to prevent all possible chances of cross contamination. The emphasis should not only be on protection but also on the cleanliness, they should be adequately cleaned and disinfected to eliminate microorganisms (Congon *et al.*, 1999). Working clothes should be cleaned every day (Bartz *et al.*, 2010) since the slaughter process can involve a lot of dirty work. In this study, however, most respondents indicated that they only wash protective clothing after 3days.

Shortcomings observed in implementation of personal hygiene practices can be addressed by proper training, educating and monitoring of the workers. Educational level and training of meat handlers regarding basic concepts of meat safety and personal hygiene plays a vital role in ensuring that the consumers are provided with safe and wholesome products (Jianu and Golet *et al.*, 2014). In addition to this regular updating and refresher courses should be carried on more frequently. This will help the meat handlers to have a better understanding of risks associated with contamination of food with microbiological pathogens and sanitation practices (McIntyre *et al.*, 2013). Most respondents in the present study from LTA: (53.33%) indicated that they did not receive training prior to employment. Both training and supervision seems to be less effective since the respondents are still engaging in poor practices. This critical violation is comparable to the proportions of respondents of other studies who also indicated that they did not receive training (Nel *et al.*, 2004; Jianu and Golet *et al.*, 2014).

Furthermore, almost half (LTA, 53.33: HTA, 50.00%) of those who indicated that they had received training only attended one training session and no refresher or updating courses were offered. It was also found that most of these respondents from LTA (46.67%) attended

the last session more than five years ago, implying that these workers may not have new and fresh knowledge regarding meat safety practices. On the other hand the Chi-square tests performed revealed professional training as a significant factor ($P < 0.05$) for knowledge, wearing gloves and reporting of illness, frequency of disinfecting equipment, working cloths and surfaces. Similar to findings of this study, Rebellato *et al.* (2012), McIntyre *et al.* (2013) and Jianu and Golet *et al.* (2014) also highlighted that individuals with proper professional training regarding meat safety significantly do better practices compared to the untrained. This shows that the quality of practices is improved mainly by professional training.

A monotonic increase in meat safety knowledge can be seen increasing along the educational levels and also as the professional years of experience increases. The scores observed at a certain educational level were significantly higher compared to other inferior levels. This is in agreement with the studies by Martins *et al.* (2012), Tan *et al.* (2013) and Jianu and Golet (2014); they noted that the highest level of knowledge was significantly ($P < 0.05$) associated with workers who had better education. This also shows that the level of knowledge is improved mainly by the educational level of an individual. That is why most workers from HTA yielded better scores, since most of them (90.91%) had indicated that they had basic formal education. However, some practices have been reported to be linked mainly to individuals' attitudes and behaviors which cannot be changed by either education or training (McIntyre *et al.*, 2013). Therefore, employing workers with at least a primary education level is important and also properly training workers helps to assure good quality products to consumers.

3.5 Conclusions

This study demonstrated a significant adherence to basic hygiene practices among slaughtermen and a satisfactory level of knowledge, with workers from HTA yielding comparatively better scores. Gaps identified highlighted the necessity of proper professional training and routine medical examinations of workers coupled with health certificates. The study underlines the link between educational level and professional training on level of knowledge and personal hygiene practices regarding meat safety. Therefore, proper training, monitoring and educating slaughtermen will help to ensure that the consumers are provided with good quality wholesome meat all the times. However, further research is recommended to validate the workers' knowledge and general practices through microbiological correlation of meat samples to these practices are also necessary. Routine inspections by responsible authorities are also advisable to assess compliance with the standards and requirements for safe meat processing in abattoirs.

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Chapter 4: Microbiological quality analysis of cattle carcasses, slaughtermen hands, equipment and water used during the slaughter process from two selected abattoirs

Abstract

The study was conducted to determine the level of contamination in beef carcass, equipment, slaughtermen hands and water with *Enterobacteriaceae*, *Salmonella*, *Escherichia coli* and aerobic colony counts (ACC) at different stages during the slaughter process (skinning, evisceration, carcass slitting, inspection, washing and packing). Cattle carcasses were sampled at four sites (rump, neck, flank and brisket) from a low through put (LTA) (n= 240) and high through put (HTA) (n= 384) abattoirs. Using conventional biochemical tests, HTA yielded significantly ($P<0.05$) higher ACC (5.2 log CFU/cm²), *E. coli* (2.6 log CFU/cm²) and *Enterobacteriaceae* (2.9 log CFU/cm²) carcass mean scores than LTA after skinning and evisceration. Washing with water in both abattoirs did not cause any significant ($P>0.05$) changes in the mean log *E. coli*, ACC and *Enterobacteriaceae*. However, there was a slight increase in microbial counts. *Salmonella* was not detected at all sampled slaughter stages. Certain abattoir-specific differences in hygiene were noted from washed and chilled carcasses. In addition, the equipment bacterial load ranged from 10 to 4 CFU/cm² for LTA, whereas in HTA it ranged from 7 to 3 CFU/cm². The counts for slaughtermen hands ranged from 15 to 8 CFU/cm² in HTA and from 10 to 5 CFU/cm² in LTA. Overall, 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. Therefore, these findings show that the equipment, slaughtermen hands and water can be sources of contamination during the slaughter process.

Keywords: *E. coli*, *Enterobacteriaceae*, hygiene, slaughter equipment, slaughter process

4.1 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 (Milios *et al.*, 2012). Based on the principles of the HACCP systems the Department of Agriculture, Forestry and Fisheries has mandated all South African registered abattoirs to use proper Hygiene Management Systems (HMS) and Hygiene Assessment Systems (HAS). All these systems are prescribed under the Meat Safety Act No. 40 of 2000 as well as Red Meat Regulations (DAFF, 2000; Government Gazette of South Africa, 2004).

For the correct 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; Milios *et al.*, 2014; Lasok and Tenhagen, 2013). 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-slaughter 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 food-borne diseases can be transferred from initial sources to the carcasses. 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. On the other hand, Buncic (2006) reported that healthy animals can still be carriers' of pathogenic bacteria and the meat can also be contaminated with fecal material during evisceration. Other sources of contamination include the equipment, water, contact surfaces and slaughtermen (Nel *et al.*, 2004; Govender *et al.*, 2013; Abd-Elaleem *et al.*, 2014). 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) 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 for the microbiological quality of meat, equipment, slaughtermen hands and water used during the slaughter process. This information is very important since the contamination of the final end product correspond 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). Findings from this study can also act as a the foundation for the development of acceptable microbiological standards or guidelines for South African abattoirs and help to develop more effective meat inspection programs in order to prevent contamination. 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 carcass at different slaughter process stages in two selected abattoirs in Eastern Cape Province. Additionally, the quantitative relationship between the cattle carcass, slaughtermen hands, equipment and water microbiological quality was also assessed. It was also hypothesized that the microbiological quality of cattle carcasses, slaughtermen hands, equipment and water used during the slaughter process from two selected abattoirs in Eastern Cape Province, is the same.

4.2 Materials and methods

4.2.1 Abattoir and slaughter processes

The study site and carrying capacities of the abattoirs are as described in Section 3.2.2. 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 and 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 approximately 16 to 20 hours at 5-7°C before distribution to respective outlets.

4.2.2 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 4.1.

Table 4.1: 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)

Bovine carcass units were selected randomly. On the other hand, the sampling sites were selected according to ISO 17604, (2003) and European Union (EU) Directive 2001/471/EC guidelines (DAFF, 2010). A total of 100cm² 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 was 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 (100cm²) for 30 seconds. Samples were packed and labelled 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 aerobic colony count (ACC), *Enterobacteriaceae*, *Escherichia coli* and *Salmonella spp.*

4.2.4 Microbiological analyses

4.2.5 Aerobic colony counts (ACC) and *Enterobacteriaceae*

Aerobic colony counts (ACC) 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-pepton, 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 1ml was placed on to the plate count agar for aerobic colony counts enumeration. The plates were incubated for 48 hours at 35°C. Presumptive colonies were counted using a Colony Counter- Digital machine (3 x magnifier, White LED array, Lasec South Africa). For *Enterobacteriaceae* enumeration, 1ml of the sample was placed on to the violet red bile glucose agar (VRBG). The plates were incubated for 24 hours at 37°C. Colonies seen to be pink to red or purple were selected and biochemical conformation tests were performed in accordance with international standards (ISO 21528-2, 2004).

4.2.6 *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 1ml of the sample to 9ml of the Ringer solution. Tenfold serial dilution for each swab sample for up to 10^{-4} were prepared and incubated at 44 °C for 48 hours. After incubation, recorded gas production from the samples as *E. coli* positive. The MPN of *E.coli* per mililitre 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 37 °C for 24 hours and examined for typical smooth pinkish colonies. Transferred 0.1ml of brilliant green broth from tubes with gas

formation into 10ml of trytone water and incubated for 48 hours at 44 °C. Determined indole production by adding Kovac's reagent to the tryptone water.

4.2.7 *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 37°C for 18hours. Using a micro-pipette, 0.1ml of the pre-enriched broth was transferred to 10ml semi-solid Rappaport-Vassiliadis soy peptone (RVS) broth. The inoculated RVS plate was incubated at 41.5°C for 24 hours. The enriched solutions were streaked onto Brilliant green agar and Xylose-lysine-deoxycholate agar and incubated at 36° C for 24 hours. Presumptive positive results were confirmed using biochemical tests according to international standards (ISO 6579, 2002).

4.2.8. Water sample collection and analysis

Water samples were collected from the primary (tank reservoir) and secondary (taps) sources. The water was collected using 500ml sterile glass bottles which were then aseptically sealed and labelled 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, 100ml of water sample was poured onto a filter paper (pore size 0.44µm) 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 35°C for 48 hours. *Escherichia coli* and coliform bacteria enumeration was done simultaneously; 100ml 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 hours at 35°C. Dark-blue to violet colonies in EMB were counted as presumptive *E. coli* and salmon to red as coliforms. *Escherichia 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 37°C for 48 hours. Black colonies after incubation were counted as presumptive *Enterococci*.

4.2.9 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 equipments sampled include knives, saws as well as meat handlers' hands. Agar contact plates were used had an internal diameter of 5.0cm. The dishes had a contact surface of 20cm², filled with violet red bile glucose agar and the others with plate count agar. They were pressed onto each sampling site for 10 seconds and correctly sealed. The plates were transported to the laboratory in a cooler bag and aerobically incubated at 37°C for 24 hours for evidence of microbial growth (ISO, 6579, 2002).

4.2.10 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± standard errors of the mean. 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, 2009). 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 (dependent 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

4.3 Results

4.3.1 Aerobic colony counts (ACC) from carcasses at different stages during slaughter

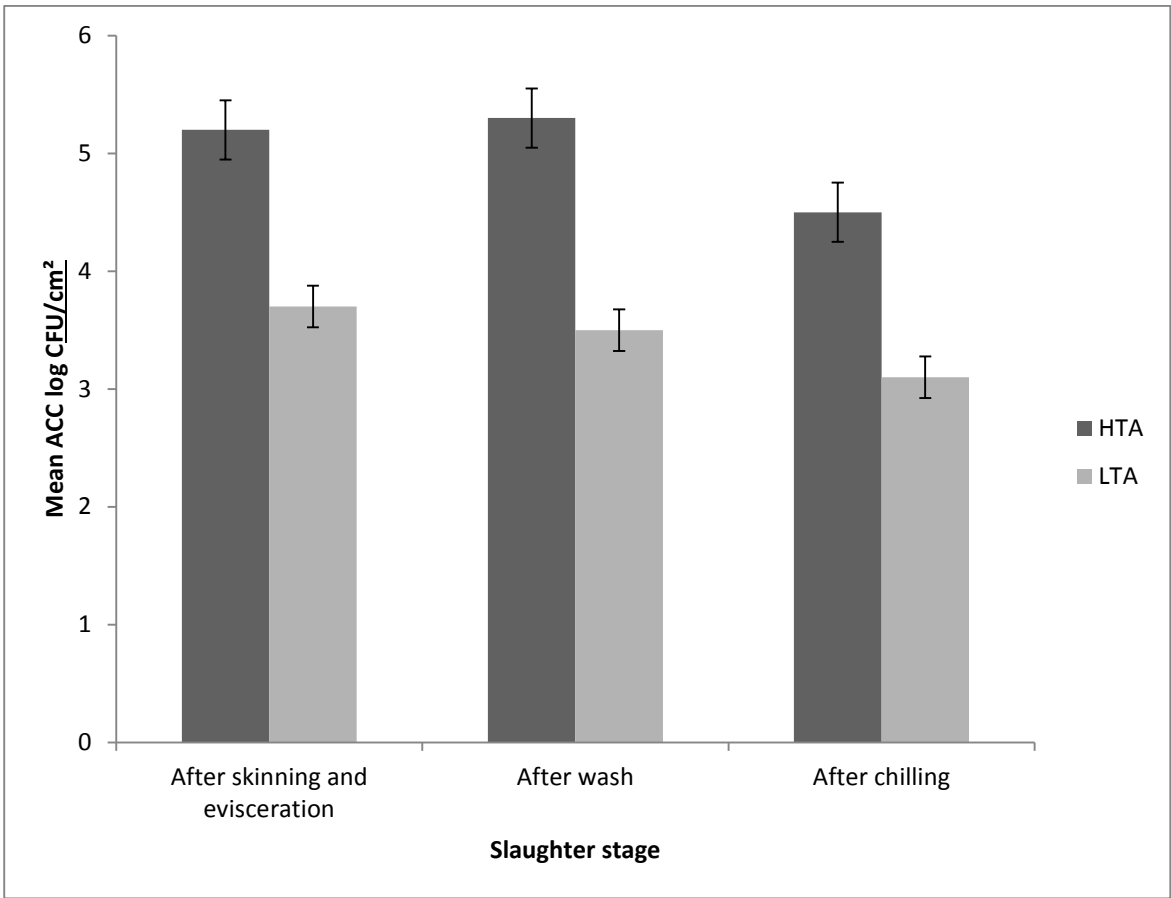
Generally, the mean log for ACC was observed to decreasing along the slaughter line, that is, from the dirty area to the clean area. For the low throughput abattoir (LTA), after skinning and evisceration the mean log was 3.7 log CFU/cm². Washing the carcasses resulted in a slight decrease of about 0.2 log CFU/cm² in ACC mean 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 high throughput abattoir (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 (Figure 4.1).

4.3.2 *E. coli*, *Salmonella* spp and *Enterobacteriaceae* from carcasses at different stages along the slaughter line

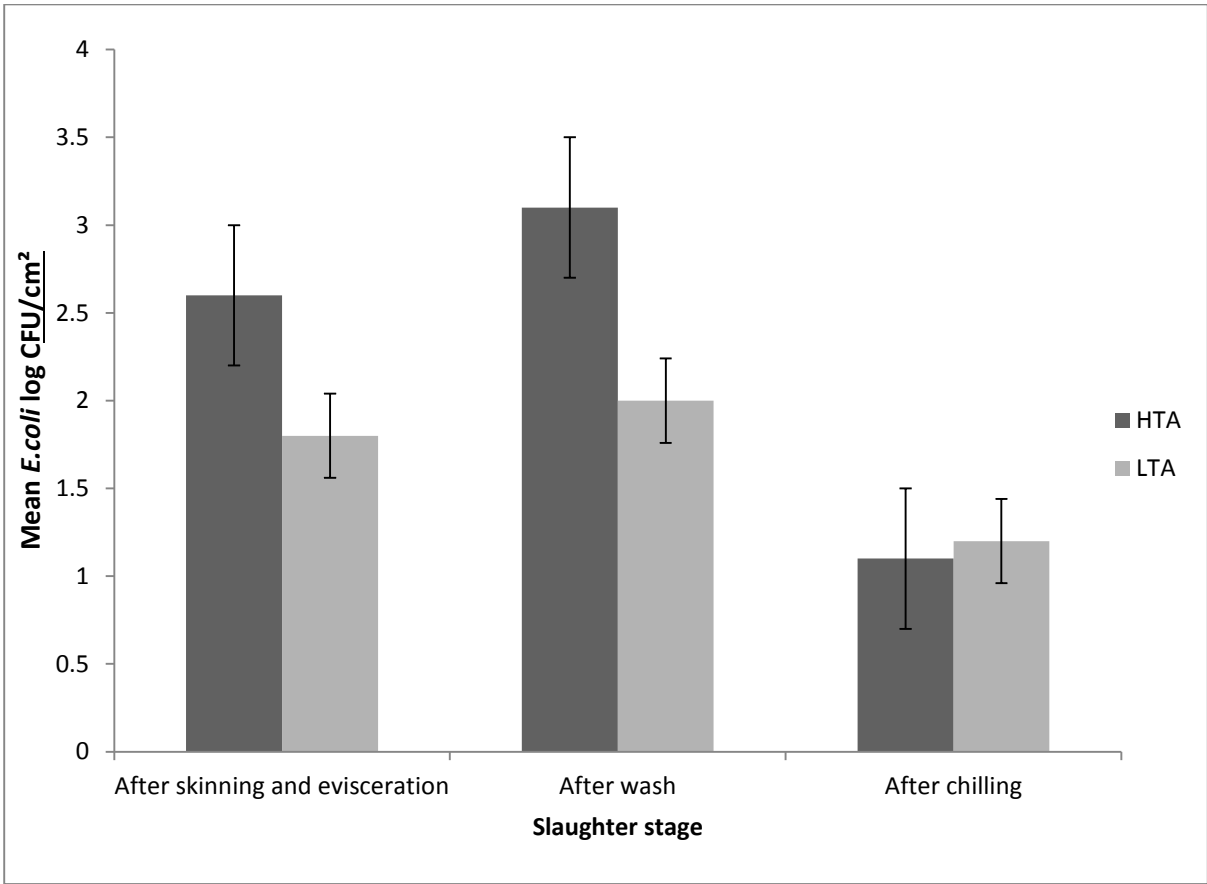
The mean log *E. coli* at different sampling stages in the LTA and HTA is presented in Figure 4.2. After skinning and evisceration the mean log was 3.4 log CFU/cm² in LTA and 5.1 log CFU/cm² in HTA. The HTA clearly yielded higher and significantly ($P < 0.05$) different mean scores than LTA. On the other 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 (Figure 4.2). *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 4.2. 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 noted.



LTA-Low throughput abattoir; *HTA- High throughput abattoir

Figure 4.1: Mean log₁₀ of Aerobic colony counts (ACC) after slaughter, evisceration, wash and chilling on carcass surfaces, at $\alpha= 0.05$



*LTA-Low throughput abattoir; *HTA- High throughput abattoir

Figure 4.2: Mean log₁₀ of *Escherichia coli* counts after slaughter, evisceration, wash and chilling on carcass surfaces, at $\alpha= 0.05$

Table 4.2: Least square means (log) \pm standard errors of *Enterobacteriaceae* counts after slaughter, evisceration, wash and chilling on carcass surfaces

Abattoir	Number of carcasses	Number of swab samples	Stage	Log CFU/cm² (Mean\pmSE)
High throughput abattoir	32	384	After Skinning and evisceration	2.9 ^a \pm 0.22
	32	384	After washing	3.6 ^a \pm 0.22
	32	384	After chilling	0.2 ^b \pm 0.31
Low throughput abattoir	20	240	After Skinning and evisceration	1.5 ^c \pm 0.26
	20	240	After washing	1.3 ^c \pm 0.26
	20	240	After chilling	1.3 ^c \pm 0.26

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

4.3.3 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 4.3. The ACC log mean showed some significant ($P < 0.05$) difference between the two abattoirs. Although day three and four 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 two (1.3 log CFU/cm²) were 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 4.3).

Water samples from both abattoirs showed significantly ($P < 0.05$) higher microbial total viable count (TVC) regardless of the source of collection (Table 4.4). *Escherichia 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, number of micro-organisms increased from the reservoir to the point of use.

4.3.4 Bacteria profile of slaughtermen hands and equipment used during the slaughter process

Evaluation of slaughtermen hands (Figure 4.3) and equipment (Figure 4.4) 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. 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 abattoirs.

Table 4.3: Least square means (\log_{10}) and \pm 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 \pm SE)	<i>E. coli</i> Log CFU/cm ² , (Mean \pm SE)	<i>Enterobacteriaceae</i> Log CFU/cm ² , (Mean \pm SE)
Low throughput abattoir	30	384	1	3.5 ^a \pm 0.41	2.0 ^a \pm 0.19	0.9 ^a \pm 0.21
	30	384	2	3.5 ^a \pm 0.41	1.3 ^b \pm 0.19	1.8 ^b \pm 0.21
High throughput abattoir	48	240	3	5.1 ^b \pm 0.09	2.5 ^a \pm 0.17	2.7 ^c \pm 0.20
	48	240	4	4.9 ^b \pm 0.09	2.1 ^a \pm 0.17	1.8 ^b \pm 0.20

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

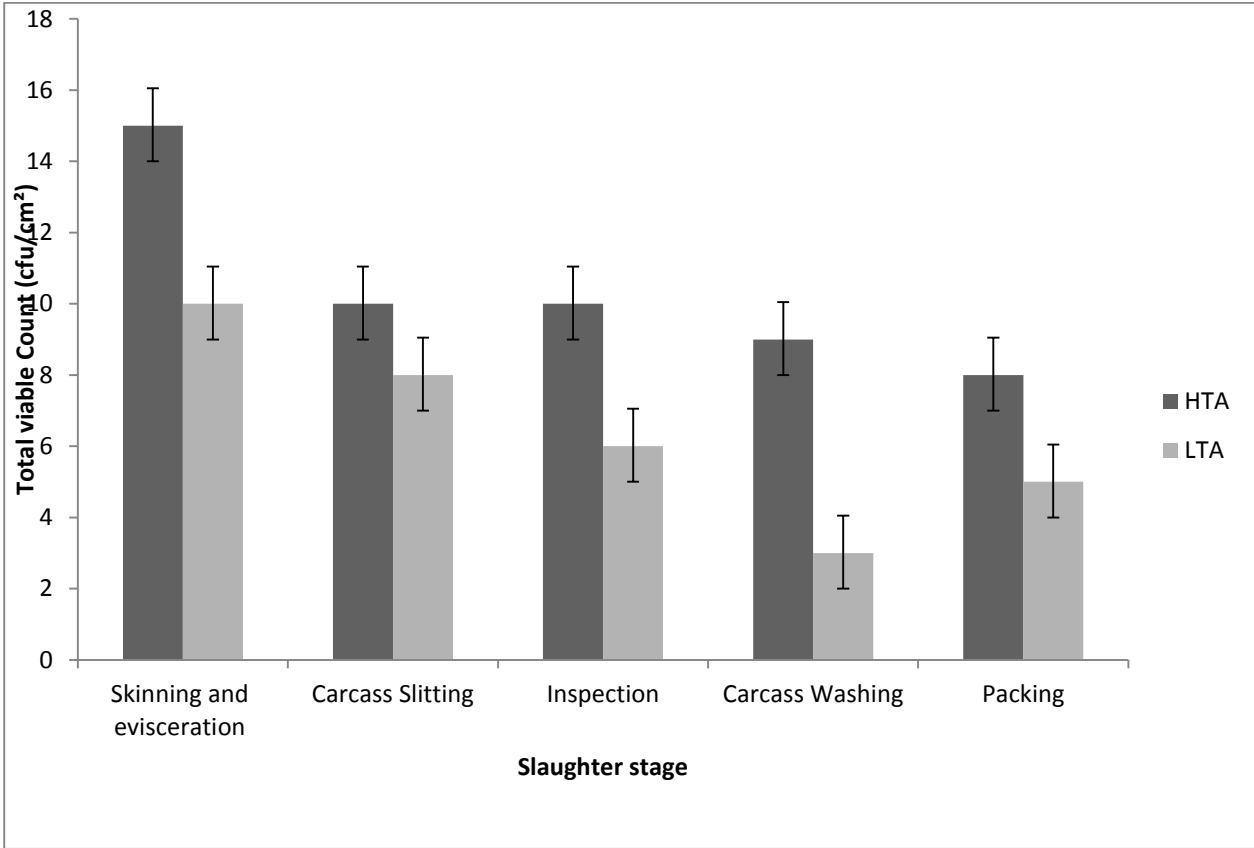
Table 4.4: Bacterial profile (CFU/100 ml) of water from the reservoir tank and tap sources used for carcass washing in abattoirs

Abattoir	Number of samples	Source	TVC CFU/ml, (35°C,48h)	Total Coliform MAC/100ml (35°C,24h)	<i>E.coli</i> MAC/100ml (35°C,24h)	<i>E.coli</i> MAC/100ml (44°C,24h)	<i>Enterococci</i> MAC/100ml (35°C,24h)
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

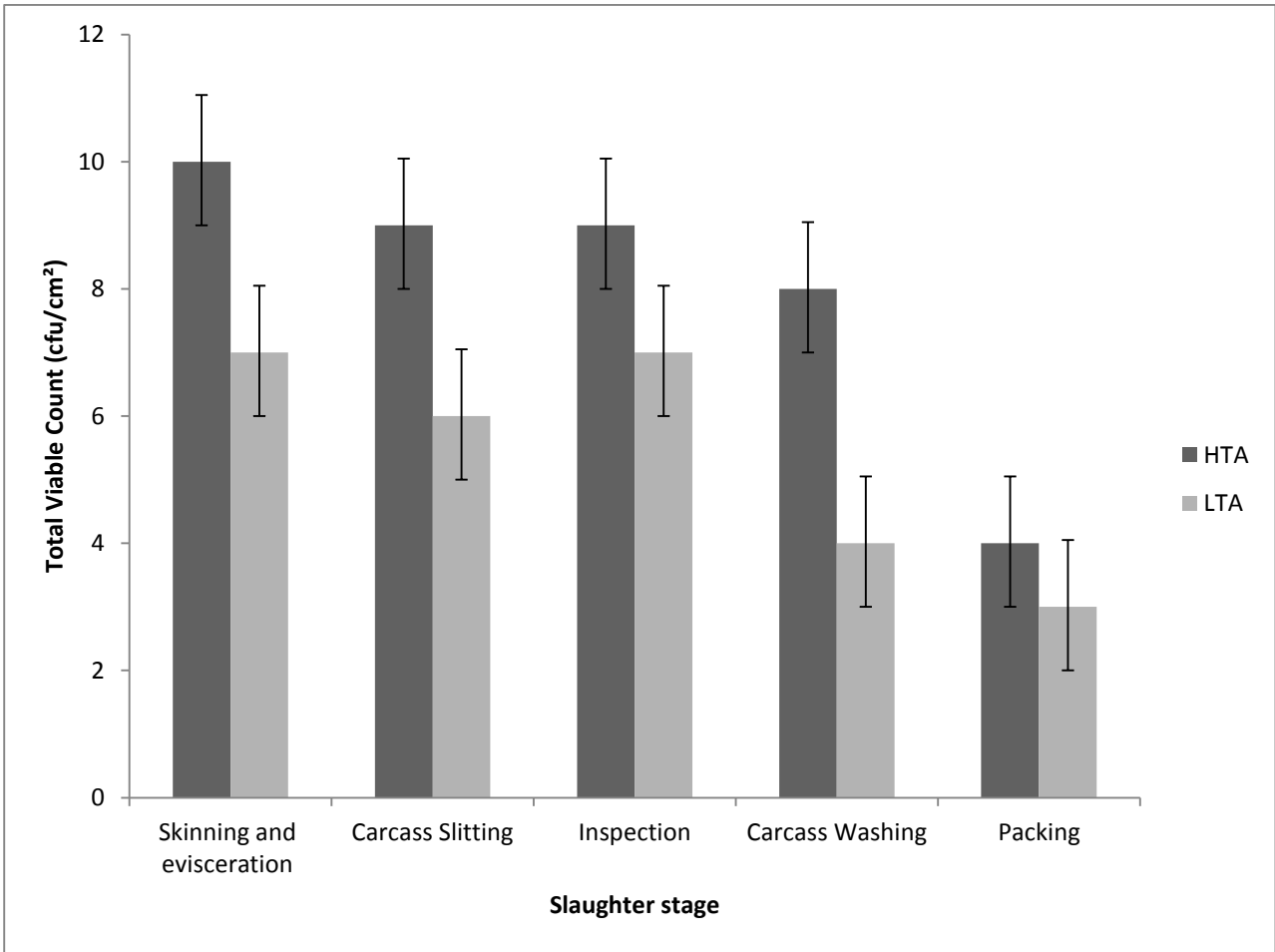
Coliform = *E. coli* like bacteria

MAC= Maximum admissible concentration



*LTA-Low throughput abattoir; *HTA- High throughput abattoir

Figure 4.3: Mean log₁₀ of Total Viable Count (TVC) at different stages during slaughter for slaughtermen hands, error bars represent 95% confidence intervals



LTA-Low throughput abattoir; *HTA- High throughput abattoir

Figure 4.4: Mean log₁₀ of Total Viable Count (TVC) at different stages during slaughter for slaughter equipment, error bars represent 95% confidence intervals

4.4 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 CFU/cm²), *E. coli* (2.6 log CFU/cm²) and *Enterobacteriaceae* (2.9 log CFU/cm²) carcass mean scores than LTA. This is in contrast to previous reports by Bell (1997), Qiongzen *et al.* (2004) and Zweifel *et al.* (2014) in which the counts did not exceed 2.0 log CFU/cm². However, they were still within the acceptable European Microbiological Standards (EC, 2005) and also in line with those reported by McEvory (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 re-distribution of the pathogens. This is in agreement with other reports by Bello (2011), McEvory (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 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. Total viable counts 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 Pittet (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).

4.5 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.

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Chapter 5: General Discussion, Conclusions and Recommendations

5.1. General Discussion

Safety control in abattoirs is very important since it is a highly labour intensive working environment which involves many workers handling carcasses at different stages. Contamination of meat usually occurs during skinning and evisceration and other sources of contamination during the slaughter process are the equipment, water, contact surfaces and personnel (Nel *et al.*, 2004; Govender *et al.*, 2013). As a result, meat processing plants have to adhere to proper hygiene practices during processing in order to assure consumer health protection and meat quality. The Department of Agriculture, Forestry and Fisheries have mandated all South African registered abattoirs to use proper Hygiene Management Systems (HMS) and Hygiene Assessment Systems (HAS). All these systems are prescribed under the Meat Safety Act No. 40 of 2000 as well as Red Meat Regulation No. 1077 of 2004 (DAFF, 2000; Government Gazette of South Africa, 2004).

The main objective of the study was to determine the microbiological quality of cattle carcasses, slaughtermen hands, equipment, and water used during the slaughter process from two selected abattoirs. Also assessed the meat safety knowledge, attitudes and handling practices of the slaughtermen in order to identify possible modes of contamination during the slaughter process. In Chapter 3, interviewed slaughtermen showed a significant adherence to basic hygiene practices and a satisfactory level of knowledge, with workers from a HTA yielding comparatively better scores. Gaps identified highlighted the necessity for proper professional training and routine medical examinations for meat handlers. These findings are in line with other studies by Nel *et al.* (2004), Abd-Elaleem *et al.* (2014), Jianu and Golet *et al.* (2014) who also identified similar gaps. In addition, the study also underlined the link between educational level and professional training on level of knowledge and personal hygiene practices regarding meat safety.

In Chapter 4, microbial counts on 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. Therefore, assessment of the slaughter process is very important since the contamination of the final end product correspond to the combined contribution of different factors during processing (Gill *et al.*, 2003; Niyonzima *et al.*, 2013; Zweifel *et al.*, 2014). Slight increase in microbial counts after washing and chilling were also noted. This can be attributed to the use non-portable water to wash carcasses as well as poor chilling methods. Total viable counts on hands and equipment were also reported to be higher though they were within the European Standards limits (EC, 2005). This implies that sterilizers are not being used as recommended. On the other hand, the identified differences in microbial counts among the sampled days can be due to different HMS implementation in these abattoirs and animal management practices at the farm (Callaway *et al.*, 2003, LeJeune *et al.*, 2004 and Buncic *et al.*, 2014). For all the sampled carcasses, equipment and slaughtermen hands, HTA yielded comparatively higher bacterial counts than the LTA. *Salmonella* was not detected at all sampled slaughter stages.

The current study has given an insight of the effectiveness of HMS in these abattoirs. This marks the first step in the development of acceptable microbiological standards or guidelines for South African abattoirs. Since visual assessment only cannot detect the most important pathogenic microorganisms like *E. coli*, *Salmonella spp*, *Listeria spp* and *Campylobacter* (Hill *et al.*, 2013). Though it might not be practical to test all products before distribution this has been reported as an effectiveness measure of hygiene in abattoirs (Kramer, 2000; Algino *et al.*, 2009; Lasok and Tenhagen, 2013).

5.2. Conclusions, Recommendations and Further research

Based on the reported changes in microbial counts on the cattle carcasses, it can be concluded that the equipment, water and slaughtermen plays a role in meat contamination during the slaughter process. The results from the interviewed slaughtermen showed a significant adherence to basic hygiene practices. The level of knowledge and handling practices of respondents were significantly different according to educational level and professional training. However, meat safety knowledge gaps identified among the slaughtermen raise the necessity for proper professional training and monitoring. This warrants further investigations to assess how training is conducted in order to identify the actual causes of poor performance by these workers. For all the sampled carcasses, equipment and slaughtermen hands, HTA yielded comparatively higher bacterial counts than the LTA. There were also significant differences in microbial counts among the sampled days in both abattoirs. This could be due to different animal management practices at the farm and abattoir hygiene. Slight increases in counts after carcass wash were noted, therefore, water might have contributed to the increase in carcass microbial counts due to use of non-potable water. On the other hand, chilling significantly reduced ACC, *E. coli* and *Enterobacteriaceae* counts on carcasses in both abattoirs. *Salmonella* was not detected at all sampled slaughter stages. Moreover, slaughtermen hands and equipment yielded more numbers of TVC than expected contributing to higher contaminations on the carcasses. Therefore, good hygiene management practices and standard evaluation measures should be employed in abattoirs which include use of potable water and proper chilling methods. However, further studies are recommended to determine the genotypic relationship among isolates from the carcasses, equipment, slaughtermen hands and water.

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Appendices

Appendix 1: Evaluation of meat safety knowledge, personal hygiene, attitudes and handling practices of slaughtermen from two selected abattoirs

This survey is conducted to gather information on **meat safety knowledge, personal hygiene, attitudes and handling practices of meat handlers from two selected abattoirs.**

This will help meat handlers from the Eastern Cape in South Africa to have a better knowledge on meat safety so that they provide consumers with safe, healthy and acceptable meat. All information provided by interviewee will be treated as STRICTLY CONFIDENTIAL for the benefit of both the researcher and respondent.

Consent

I hereby agree to participate in this survey regarding the **meat safety knowledge, general hygiene and handling practices of meat handlers from abattoirs.** I participated freely without being forced and I was free to stop at any point if I was not willing to continue. I also understand that any of my decisions will not be used against me or affect me negatively.

I understand that this is a research project whose purpose is not necessarily to benefit me personally and my answers will remain confidential.

Signature of participant.....

Date.....

Section 1 Demographic information: Tick the appropriate box

Gender	Male	Female				
Age	≤20	21-30	31-40	41-50	>50	
Marital status	Single	Married	Divorced	Widow	Widower	
Education level	None	Primary	Secondary		Tertiary	
Tribe	Xhosa	Zulu	Coloured		Others (specify)	
Religion	Christianity	Traditional			Other (specify)	

Section 2 Premises and personal information: Tick were appropriate.

- For how long have you been in the meat industry (experience)?

<1year.....2-5years.....>5 years.....

- Did you receive formal training in environmental sanitation, meat safety and hygiene before you started working

Yes.....No.....

- How many times have you received training?

One training session.....2-5 training sessions.....6 and more training sessions.....Never.....

➤ When was your last training session?

Less than 6months ago.....1-2years ago.....more than 2 years ago.....Never.....

➤ Do you have a valid health certificate

Yes.....No.....

➤ How often do you go for medical examination

Every month.....After 6 months.....Annually.....No need.....

Section 3 Food-borne diseases awareness: Tick were appropriate.

➤ Do you know that food can cause diseases?

Yes.....No.....Don't know.....

➤ Do you have any experience with food-borne diseases?

Yes.....No.....

➤ Food-borne diseases that you know?

Diarrhea.....Typhoid.....Cholera.....Other.....No idea.....

➤ Signs and symptoms of food-borne diseases that you know?

Vomiting.....Abdominal pains.....Frequent stooling.....Nausea.....Fever..... Headaches.....Other.....

➤ Do you come to work or report when you show the above signs and symptoms?

Yes.....No.....Sometimes.....

- How do you seek medication when you show the above signs and symptoms?

Self-medication.....Traditional medicine.....Go to pharmacy
for drugs.....Go to the hospital for diagnosis and treatment.....

Section 4 Personal hygiene: Tick were appropriate.

- Do you remove personal belongings (jewellery, mobile phone, etc.) before handling meat

Yes.....No.....Occasionally.....

- Do you smoke or eat during work?

Yes.....No.....Sometimes.....

- How often do you clean hands before handling or serving customers?

Always.....Occasionally.....Never.....

- How do you clean your hands?

Using water only.....Hand sanitizer.....Soap and cold water.....Soap
and hot water.....Other.....

- How do you dry your hands?

Using disposable paper towels.....Cloth towel.....Do not
dry.....

- Why do you wash hands?

To kill pathogens.....To prevent contamination of food.....I don't
know.....

➤ How often do you clean and disinfect equipment (knives, saws etc) during the slaughter process.

Always.....Sometimes.....Never.....

➤ How often do you clean and disinfect serving contact surfaces?

Always.....Sometimes.....Never.....

➤ How often do you clean and disinfect working clothes (visually clean)?

Daily.....After 2days.....After 3days.....After a week.....Other.....

➤ How often do you wear protective clothing

• Apron- Always.....Sometimes.....Never.....

• Overalls- Always.....Sometimes.....Never.....

• Hairnets- Always.....Sometimes.....Never.....

• Beard nets- Always.....Sometimes.....Never.....

• Gumboots- Always.....Sometimes.....Never.....

• Gloves- Always.....Sometimes.....Never.....

Appendix 2: Ethical clearance certificate



University of Fort Hare
Together in Excellence

ETHICAL CLEARANCE CERTIFICATE REC-270710-028-RA Level 01

Certificate Reference Number: MUS051SNYA01

Project title: **Evaluation of meat safety knowledge among meat handlers and microbiological analysis of meat from abattoirs, retailers and informal trades in Amathole district, Eastern Cape.**

Nature of Project: Masters

Principal Researcher: Faith Nyamakwere

Supervisor: Dr B Mushonga
Co-supervisor: Prof V Muchenje

On behalf of the University of Fort Hare's Research Ethics Committee (UREC) I hereby give ethical approval in respect of the undertakings contained in the above-mentioned project and research instrument(s). Should any other instruments be used, these require separate authorization. The Researcher may therefore commence with the research as from the date of this certificate, using the reference number indicated above.

Please note that the UREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the document
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research

The Principal Researcher must report to the UREC in the prescribed format, where applicable, annually, and at the end of the project, in respect of ethical compliance.

Special conditions: Research that includes children as per the official regulations of the act must take the following into account:

Note: The UREC is aware of the provisions of s71 of the National Health Act 61 of 2003 and that matters pertaining to obtaining the Minister's consent are under discussion and remain unresolved. Nonetheless, as was decided at a meeting between the National Health Research Ethics Committee and stakeholders on 6 June 2013, university ethics committees may continue to grant ethical clearance for research involving children without the Minister's consent, provided that the prescripts of the previous rules have been met. This certificate is granted in terms of this agreement.

The UREC retains the right to

- Withdraw or amend this Ethical Clearance Certificate if
 - Any unethical principal or practices are revealed or suspected
 - Relevant information has been withheld or misrepresented
 - Regulatory changes of whatsoever nature so require
 - The conditions contained in the Certificate have not been adhered to
- Request access to any information or data at any time during the course or after completion of the project.
- In addition to the need to comply with the highest level of ethical conduct principle investigators must report back annually as an evaluation and monitoring mechanism on the progress being made by the research. Such a report must be sent to the Dean of Research's office

The Ethics Committee wished you well in your research.

Yours sincerely


Professor Gideon de Wet
Dean of Research

23 October 2014